
British Journal of

Venereal Diseases

British Journal of Venereal Diseases

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ENG-406334-1- p25894

British Journal of Venereal Diseases

Edited for the Medical Society for the Study of Venereal Diseases

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VOLUME 55, 1979

BRITISH MEDICAL ASSOCIATION

TAVISTOCK SQUARE, LONDON WC1H 9JR

P 25,894

Contents

February 1979 Vol. 55 No. 1

An assessment of the Microcult-GC culture test

P. F. UNSWORTH, H. TALSANIA, AND I. PHILLIPS (Gt Britain) *page 1*

Serum immunoglobulin response in uncomplicated gonorrhoea

A. McMILLAN, GILLIAN McNEILLAGE, H. YOUNG, AND SHEILA R. BAIN (Gt Britain) *page 5*

Diagnosis of gonorrhoea in women

R. N. THIN AND ELIZABETH J. SHAW (Gt Britain) *page 10*

Pharyngeal colonisation by *Neisseria gonorrhoeae* and *Neisseria meningitidis* in black and white patients attending a venereal disease clinic

R. C. NOBLE, R. M. COOPER, AND BETTY R. MILLER (USA) *page 14*

Inhibition of *Neisseria gonorrhoeae* by normal human saliva

A. MELLERSH, A. CLARK, AND S. HAFIZ (Gt Britain) *page 20*

Short-term treatment of gonorrhoea with intramuscular and oral forms of trimethoprim-sulphamethoxazole

A. LASSUS AND O.-V. RENKONEN (Finland) *page 24*

Antibodies to *Chlamydia trachomatis* in acute salpingitis

J. D. TREHARNE, K. T. RIPA, P.-A. MÅRDH, L. SVENSSON, L. WESTRÖM, AND S. DAROUGAR

(Gt Britain and Sweden) *page 26*

Ureaplasma urealyticum and *Mycoplasma hominis* in chlamydial and non-chlamydial nongonococcal urethritis

D. TAYLOR-ROBINSON, R. T. EVANS, E. D. COUFALIK, M. J. PRENTICE, P. E. MUNDAY, G. W. CSONKA, AND J. K. OATES

(Gt Britain) *page 30*

Treatment of nongonococcal urethritis with rifampicin as a means of defining the role of *Ureaplasma urealyticum*

E. D. COUFALIK, D. TAYLOR-ROBINSON, AND G. W. CSONKA (Gt Britain) *page 36*

Herpesvirus hominis antibodies among children and young adults in Ibadan

A. O. SOGBETUN, D. MONTEFIORE, AND C. N. ANONG (Nigeria) *page 44*

Necrotising balanitis due to a generalised primary infection with herpes simplex virus type 2

J. F. PEUTHERER, ISABEL W. SMITH, AND D. H. H. ROBERTSON (Gt Britain) *page 48*

Current approaches to the diagnosis, treatment, and reporting of trichomoniasis and candidosis

B. H. O'CONNOR AND M. W. ADLER (Gt Britain) *page 52*

A theory of the epidemiology of gonorrhoea

W. F. FELTON (Gt Britain) *page 58*

Treatment failure with penicillin in early syphilis

A. J. H. GILES AND A. G. LAWRENCE (Gt Britain) *page 62*

Correspondence *page 65*

Notice *page 67*

Report of the Medical Society for the Study of Venereal Diseases *page 68*

Abstracts *page 70*

April 1979 Vol. 55 No. 2

Bulletin of the International Union against the Venereal Diseases and Treponematoses: 29th General Assembly, Leeds, 21-25 May 1978 *page 75*

Theme 1: Recent advances in the microbiology and immunology of gonorrhoea

Sensitivity of *Neisseria gonorrhoeae* to penicillin in West Germany D. PETZOLDT, K. GRUNDER, AND U. NEUBERT (West Germany) *page 80*

Effect of different preparation procedures on the ultrastructure of gonococci W. GEBHART, W. JURECKA, J. SÖLTZ-SZÖTS, J. THURNER, A. ELLINGER, AND K. KITZ (Austria) *page 83*

Preservation of *Neisseria gonorrhoeae* by the gelatin-disc method S. YAMAI, Y. OBARA, T. NIKKAWA, Y. SHIMODA, AND Y. MIYAMOTO (Japan) *page 90*

Theme 2: Recent advances in the immunology of syphilis

Role of humoral versus cellular mechanisms of resistance in the pathogenesis of syphilis M. METZGER (Poland) *page 94*

Attenuated yaws in Surinam P. L. A. NIEMEL, E. A. BRUNINGS, AND H. E. MENKE (Surinam) *page 99*

Comparison of cardiolipin and treponemal tests in the serodiagnosis of yaws H. E. MENKE, J. VELDKAMP, E. A. BRUNINGS, P. L. A. NIEMEL, A. NOTOWICZ, AND E. STOLZ (The Netherlands) *page 102*

Double-staining procedure for the FTA-ABS test E. F. HUNTER, R. M. MCKINNEY, S. E. MADDISON, AND D. D. CRUCE (USA) *page 105*

A note on microscopy F. J. AUMONIER (Gt Britain) *page 109*

Therapeutic effect of oral doxycycline on syphilis Y. ONODA (Japan) *page 110*

Theme 3: Recent advances in the study of STDs due to viral and other agents

Follow up of patients with hepatitis B surface antigen P. D. SIMMONS (Gt Britain) *page 116*

Group B streptococcal infection J. M. KNOX (USA) *page 118*

Vaccination for herpes simplex genitalis TH. NASEMANN AND S. W. WASSILEW (West Germany) *page 121*

Therapeutic aspects of herpes genitalis J. SÖLTZ-SZÖTS AND D. FANTA (Austria) *page 123*

Theme 4: New trends in the epidemiology of STDs

Illness behaviour and STDs R. M. HARRISON (Gt Britain) *page 125*

Critical evaluation of the venereal disease control campaign in Poland (1970-76) J. TOWPIK (Poland) *page 127*

Epidemiology of gonorrhoea and syphilis in South Australia (1966-77) D. JORGENSEN (Australia) *page 131*

Epidemiology of gonorrhoea and syphilis in New Zealand W. M. PLATTS (New Zealand) *page 138*

Does gonorrhoea surveillance pay? R. C. RENDTORFF AND H. PACKER (USA) *page 142*

An alternative to the problem of insufficient physician manpower in the clinical care of STDs C. W. PARTON AND S. M. SGROI (USA) *page 144*

Results of routine screening for STDs in Austria over a six-year period D. FANTA, W. GEBHART, W. GROSS, E. M. KOKOSCHKA, J. SÖLTZ-SZÖTS, AND J. THURNER (Austria) *page 147*

Epidemiological importance of concealed nongonococcal urethritis R. R. WILLCOX (Gt Britain) *page 149*

Abstracts *page 154*

Notice *page 159*

Correction *page 159*

June 1979 Vol. 55 No. 3

The Harrison Lecture, 1978: Sexually transmitted diseases and travel
C. S. NICOL (Gt Britain) *page 161*

Ultrastructural study of satellite lymph nodes in syphilitic rabbits

R. W. LAPUSHIN, R. E. BAUGHN, D. M. MUSER, AND P. GYORKEY (USA) *page 168*

Treatment monitoring in syphilis using the autoanalyser

D. E. MacFARLANE AND A. McMILLAN (Gt Britain) *page 173*

Rapid presumptive diagnosis of gonococcal urethritis in men by the limulus lysate test
V. A. SPAGNA, R. B. PRIOR, AND R. I. PERKINS (USA) *page 179*

Assessment of a selective medium for the isolation of *Neisseria gonorrhoeae*
E. TAYLOR AND I. PHILLIPS (Gt Britain) *page 183*

Comparison of anal and rectal swabs in the diagnosis of anorectal gonorrhoea in women
B. KOLATOR AND P. RODIN (Gt Britain) *page 186*

Individual susceptibility to neisserial infection?
H. YOUNG, A. B. HARRIS, AND D. H. H. ROBERTSON (Gt Britain) *page 188*

Mezlocillin in the treatment of gonorrhoea
A. TASSUS AND O.-V. RINKONEN (Finland) *page 191*

Growth and effect of chlamydiae in human and bovine oviduct organ cultures
G. R. HUTCHINSON, D. TAYLOR-ROBINSON, AND R. R. DOORMASHKIN (Gt Britain) *page 194*

Chlamydia trachomatis in acute salpingitis
I. PAAVONEN, P. SAIKKU, E. VESTERINEN, AND K. AHO (Finland) *page 203*

HLA and sexually transmitted diseases in prostitutes
S. H. CHAN, T. TAN, A. KAMARUDIN, G. B. WEE, AND V. S. RAJAN (Singapore) *page 207*

Clinical pharmacological studies of amoxycillin: effect of probenecid
R. BARBHAIIYA, R. N. THIN, P. TURNER, AND J. WADSWORTH (Gt Britain) *page 211*

Haematuria presenting in outpatients attending a department of genitourinary medicine
K. L. AMARASURIYA (Gt Britain) *page 214*

Seasonal variations in the reported incidence of sexually transmitted diseases in Scotland (1972-1976)
C. B. S. SCHOFIELD (Gt Britain) *page 218*

Reinfection with disseminated gonococcal disease: a case report
N. J. FIUMARA AND A. D. SIMKIN (USA) *page 223*

Sexually transmitted diseases: Extract from the Report of the Chief Medical Officer of the Department of Health and Social Security, 1977 *page 225*

Correspondence *page 230*

Book reviews *page 231*

Notices *page 232*

Abstracts *page 233*

August 1979 Vol. 55 No. 4

Evaluation of microhaemagglutination assay to determine treponemal antibodies in CSF
A. BIRRY AND S. KASATIYA (Canada) *page 239*

Attachment of *Neisseria gonorrhoeae* to human sperm: Microscopical study of effect of trypsin and iron
C. I. GOMEZ, W. A. STENBACK, A. N. JAMES, B. S. CRISWELL, AND R. P. WILLIAMS (USA) *page 245*

Studies of ciliated epithelia of the human genital tract
P.-A. MÅRDH, B. BALDETORP, C. H. HÅKANSSON, H. FRITZ, AND I. WESTRÖM (Sweden) *page 256*

AUGUST 1979 Vol. 55 No. 4 *continued*

Secretory antibody response of the cervix to infection with *Neisseria gonorrhoeae*
A. McMILLAN, G. McNEILLAGE, H. YOUNG, AND S. S. R. BAIN (Gt Britain) *page 265*

Evaluation of urethral culture for *Neisseria gonorrhoeae* in the routine investigation of men attending a STD clinic
A. McMILLAN AND R. S. PATTMAN (Gt Britain) *page 271*

Gonorrhoea in women in Scotland
C. B. S. SCHOFIELD (Gt Britain) *page 274*

How often are gonorrhoea and genital yeast infection sexually transmitted?
R. N. THIN, P. RENDELL, AND J. WADSWORTH (Gt Britain) *page 278*

The need for a chlamydial culture service
I. R. WILLCOX, P. G. FISK, J. BARROW, AND D. BARLOW (Gt Britain) *page 281*

Double-blind comparison of two regimens in the treatment of nongonococcal urethritis: seven-day vs 21-day courses of triple tetracycline (Deteclo)
I. V. THAMBAR, P. D. SIMMONS, R. N. THIN, S. DAROUGAR, AND P. YEARSLEY (Gt Britain) *page 284*

Rapid staining technique for *Trichomonas vaginalis*
M. J. BALSDON, N. GREEN, C. W. ANDREW, AND D. H. JACKSON (Gt Britain) *page 289*

False-positive test results for syphilis in relatives of a patient with systemic lupus erythematosus
S. JESSOP AND P. BOTHA (South Africa) *page 292*

Social distribution of sexually transmitted diseases
P. M. BACON (Gt Britain) *page 295*

Sexually transmitted diseases in Ontario
R. D. CATTERALL (Gt Britain) *page 300*

Australian conference on STD in Perth, Western Australia, August 1978
R. S. MORTON *page 304*

Correspondence *page 305*

Book reviews *page 306*

Notice *page 306*

Abstracts *page 307*

October 1979 Vol. 55 No. 5

Study of STDs in patients attending venereal disease clinics in Khartoum, Sudan
O. M. A. TAHA, M. HAG ALI, E. E. OMER, M. A. AHMED, AND S. A. ABBARO (Sudan) *page 313*

Use of CB hamsters in the study of *Treponema pertenue*
R. F. SCHELL, I. L. LE FROCK, J. P. BABU, AND J. K. CHAN (USA) *page 316*

Cultivation of *Treponema pallidum* in subcutaneous chambers implanted in golden hamsters
G. D. MORRISON, S. I. EGGLESTON, AND I. L. NORTHWOOD (Gt Britain) *page 320*

Sensitivity of *Neisseria gonorrhoeae* to antibiotics in London (1976-78)
A. D. SETH, B. KOLATOR, AND A. E. WILKINSON (Gt Britain) *page 325*

OCTOBER 1979 Vol. 55 No. 5 *continued*

Failure of iron to promote attachment of gonococci to human spermatozoa under physiological conditions
A. P. JOHNSON AND MARY F. OSBORN (Gt Britain) *page 329*

Frequency of simultaneous carriage of *Neisseria gonorrhoeae* and *Neisseria meningitidis*
K. ØDEGAARD AND T. W. GEDDE-DAHL (Norway) *page 334*

Meningococcal colonisation misdiagnosed as gonococcal pharyngeal infection
R. C. NOBLE AND R. M. COOPER (USA) *page 336*

Double-blind trial of amoxycillin and ampicillin plus probenecid in the treatment of gonorrhoea in men
A. R. BRATHWAITE (Jamaica) *page 340*

Single-dose minocycline in the treatment of gonococcal urethritis
P. M. WATERWORTH, J. D. ORIEL, G. L. RIDGWAY, AND S. SUBRAMANIAN (Gt Britain) *page 343*

Vaginal oxytetracycline concentrations
R. N. THIN, Z. H. AL RAWI, P. D. SIMMONS, J. TREHARNE, AND S. TABAQCHALI (Gt Britain) *page 348*

Metronidazole-resistant *Trichomonas vaginalis*
A. FORSGREN AND L. FORSSMAN (Sweden) *page 351*

Double-blind comparison of a single dose and a five-day course of metronidazole in the treatment of trichomoniasis
R. N. THIN, M. A. E. SYMONDS, R. BOOKER, S. COOK, AND F. LANGLET (Gt Britain) *page 354*

Epidemiological investigation of patients with vulvovaginal candidosis
D. W. WARNOCK, D. C. E. SPELLER, J. D. MILNE, A. L. HILTON, AND P. I. KERSHAW (Gt Britain) *page 357*

Effect of simultaneous oral and vaginal treatment on the rate of cure and relapse in vaginal candidosis
J. D. MILNE AND D. W. WARNOCK (Gt Britain) *page 362*

Sexual transmission of hepatitis B surface antigen
N. INABA, R. OHKAWA, A. MATSUURA, J. KUDOH, AND H. TAKAMIZAWA (Japan) *page 366*

Staphylococcus saprophyticus in the aetiology of nongonococcal urethritis
B. HOVELIUS, I. THELIN AND P.-A. MÅRDH (Sweden) *page 369*

Epidemic of amoebiasis and giardiasis in a biased population
B. H. KEAN, D. C. WILLIAM, AND S. K. LUMINAIS (USA) *page 375*

Correspondence *page 379*

Book reviews *page 381*

Abstracts *page 382*

December 1979 Vol. 55 No. 6

Optimum concentration of dissolved oxygen for the survival of virulent *Treponema pallidum* under conditions of low oxidation-reduction potential
S. GRAVES AND T. BILLINGTON (Australia) *page 387*

Susceptibility of rabbits to *Treponema pallidum* after infection with *Mycobacterium bovis*
S. GRAVES (Australia) *page 394*

Limited protection of rabbits against infection with *Treponema pallidum* by immune rabbit sera
S. GRAVES AND J. ALDEN (Australia) *page 399*

Reverse passive haemagglutination test for the rapid identification of *Neisseria gonorrhoeae* and detection of penicillinase production

R. MUNRO AND R. MALLON (Australia) *page 404*

Prevalence of rectal and pharyngeal infection in women with gonorrhoea in Sheffield

G. R. KINGHORN AND S. RASHID (Gt Britain) *page 408*

Comparison of minocycline and ampicillin in gonococcal urethritis

M. A. WAUGH, E. MARY COOKE, B. B. G. NEHAUL, AND JANET BRAYSON (Gt Britain) *page 411*

Chlamydia trachomatis genital infections

F. D. GHADIRIAN AND H. G. ROBSON (Canada) *page 415*

Antichlamydial antibodies in pelvic inflammatory disease

P. D. SIMMONS, T. FORSEY, R. N. THIN, J. D. THREHARNE, S. DAROUGAR, F. LANGLET, AND P. K. PANDHI (Gt Britain) *page 419*

Chlamydia trachomatis infection of the Fallopian tubes

B. R. MØLLER, L. WESTRÖM, S. AHRONS, K. T. RIPA, L. SVENSSON, C. von MECKLENBURG, H. HENRIKSON AND P.-A. MÅRDH (Denmark and Sweden) *page 422*

Prevalence of nine different micro-organisms in the female genital tract

K. PERSSON, KRISTINA PERSSON, H. HANSSON, B. BJERRE, L. SVANBERG, T. JOHNSON, AND A. FORSGREN (Sweden) *page 429*

Effect of hysterectomy on genital infections

F. N. JUDSON AND M. A. RUDER (USA) *page 434*

Rising incidence of chancroid in Rotterdam

K. C. NAYYAR, E. STOLZ, AND M. F. MICHEL (Holland) *page 439*

Effect of injections of small doses of human fibroblast interferon into genital warts

G. M. SCOTT AND G. W. CSONKA (Gt Britain) *page 442*

Perigenital cutaneous schistosomiasis

F. A. B. ADEYEMI-DORO, A. O. OSOBA, AND T. A. JUNAID (Nigeria) *page 446*

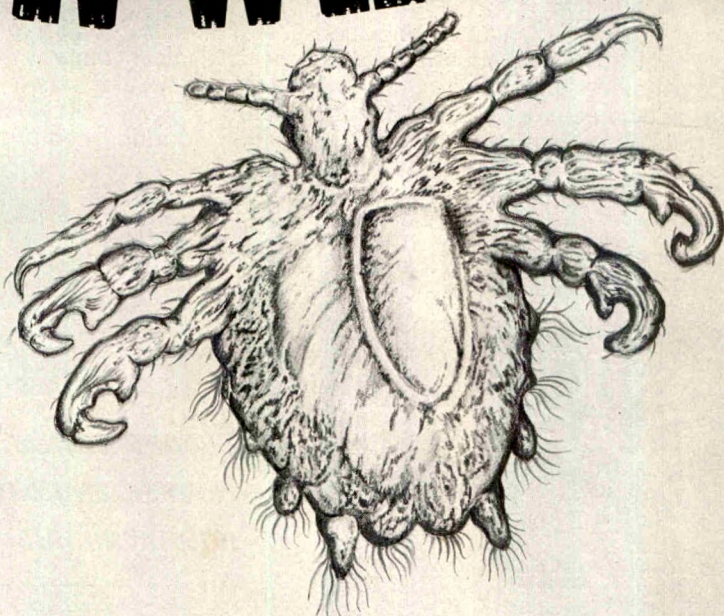
Correspondence *page 450*

Correction *page 450*

Book reviews *page 451*

Index *page 452*

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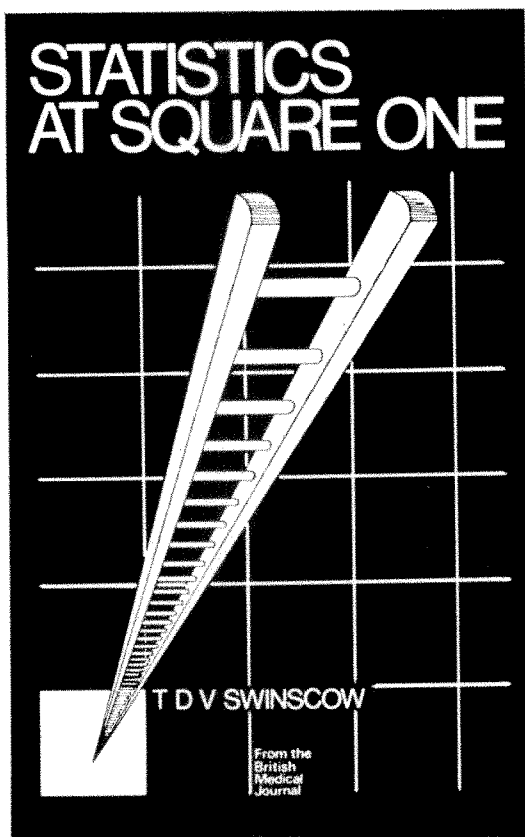
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M.S.S.V.D.

The Medical Society for the Study of Venereal Diseases

11 Chandos Street, Cavendish Square,
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President: Dr. E. M. C. Dunlop

The object of the Society is to bring together those interested or engaged in the diagnosis, treatment, and public health aspects of venereal diseases. The Society meets regularly in London for the reading of papers, discussions, and presentation of cases. An out-of-London meeting is held in the Spring.

Annual subscription for membership of the Society is £12 or \$22.00. for all members.

Applications for membership are very welcome and forms can be obtained from the Honorary Secretary, Dr. J. Barrow, Lydia Department, St. Thomas' Hospital, SE1 7EH.

Forthcoming Programme 1978

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|--------|---|
| March. | <p>Symposium on Gonorrhoea:
 "Evaluation of diagnostic tests",
 Dr. David Barlow.
 "Serological tests for gonorrhoea",
 Professor Alan Glynn.
 "Resistant strains of N. gonorrhoea",
 Professor Ian Phillips.
 "The Epidemiology of Beta-lactamase producing gonococci",
 Dr. O. P. Arya.</p> |
| April. | <p>Contact Tracing and Health Education:
 "Contact Tracing", Mrs. Isobel Hunter and Miss Hilary Keay.
 "Health Education and sexually transmitted diseases", Dr. George Cust.
 "The work of the DHSS—International Contact Tracing" by Dr. Mary Sibellas.</p> |

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Editorial

Clinic practices

Those working in clinics for the treatment of sexually transmitted diseases (STDs) have a long history of critical self-appraisal, and reports on the findings have regularly appeared in the *British Journal of Venereal Diseases*. Larger scale surveys have also been conducted by the British Co-operative Clinical Group and great credit should be paid to Dr R. R. Willcox for all the work he has done compiling reports on their activities. On page 2 we publish the first of a series of reports from Dr M. W. Adler and his colleagues from the Medical Research Council's Group on Sexually Transmitted Diseases at the Middlesex Hospital, London, on a survey that has been conducted into the work of clinics in England and Wales. This is a valuable extension of the work done by the British Co-operative Clinical Group.

This report shows the high standards of clinical care given to patients attending most of the clinics. One aspect of care does give cause for concern; in a few clinics nurses are allowed to examine, investigate, and prescribe treatment for patients after consulting with the doctor by telephone. Many of these nurses are competent and provide a good service. While this is in line with the expanding role of clinical nurses (Department of Health and Social Security, 1977), it does not necessarily accord with the provision of the highest standards of clinical care. Clinics where this occurred were open for a mean of 17 hours, compared with a mean of 14½ hours for all clinics. It might be asked if these long opening hours, some without a doctor in the clinic, are really in patients' best interests, and it is hoped that consultants in charge of these clinics are striving to obtain more doctors' services to cover the clinics. These consultants deserve the strongest support in their efforts to obtain staff. Furthermore the role of the nurse in STD clinics needs further investigation.

In countries where there is still a shortage of doctors, particularly in areas remote from centres of excellence, nurses may be the only staff available

to treat cases of STD and may supply a good service to their patients. It is hoped that in due course standards can be raised and the service improved in these areas.

In the second paper, on page 10, Dr Adler shows that the standards of diagnosis of gonorrhoea are reasonably uniform. In contrast, in the third paper, page 15, he describes the wide variety of treatments prescribed for uncomplicated gonorrhoea. This variation has been remarked on by visitors from overseas to clinics in this country and has even caused confusion to some of them. It is vitally important that those in charge of clinics monitor the results of their treatment. Provided satisfactory cure rates are maintained, and most authorities would regard cure rates for uncomplicated gonorrhoea of 95% or higher as satisfactory, this is a healthy state of affairs. This variation in treatment contrasts with more rigid recommendations in some other countries. While there are certain advantages to such recommendations, they may limit the scope for experiment, remove initiative from the treating physician, and with rapidly changing patterns of gonococcal antibiotic sensitivity patterns, they may lead to a sense of false security.

It is also hoped that the findings in Dr Adler's third paper, on page 19, will help all physicians to review their own criteria for reporting cases. The totals compiled in the United Kingdom depend on the accuracy of the returns from the clinics, and it is apparent that there is variation between clinics in some criteria.

Reference

Department of Health and Social Security (1977). *Health Services Management: The Extending Role of the Clinical Nurse—Legal Implications and Training Requirements*. Circular HC (77) 22.

Facilities and diagnostic criteria in sexually transmitted disease clinics in England and Wales

M. W. ADLER, E. M. BELSEY, B. H. O'CONNOR, R. D. CATTERALL, AND D. L. MILLER

From the Departments of Venereology and Genitourinary Medicine and Community Medicine, Middlesex Hospital and Medical School, London

SUMMARY A study was conducted to collect information from consultants about the facilities and diagnostic criteria used in clinics for sexually transmitted diseases in England and Wales. Most of the information was obtained by personal interview with a response rate of 92%. Half the clinics were open for 10 hours or less a week, the mean length of time for all clinics was 14½ hours a week. Eighty per cent of clinics had a full or part-time contact-tracing service. All the clinics had microscopical and serological services and almost all (99%) had cultural facilities. The policy concerning the most efficient use of these facilities is discussed.

Introduction

The service for sexually transmitted diseases (STDs) is unusual for three reasons. Firstly, a free and confidential service existed for over three decades before the inception of the National Health Service. Secondly, unlike any other hospital-based service, apart from Accident and Emergency, an 'open door' policy is maintained and patients are encouraged to attend without being referred by their general practitioner. Finally, the numbers of cases treated in clinics are notified to the Department of Health and Social Security (DHSS). A further important aspect is the willingness of those working in the specialty to examine their own service and clinical practices. This tradition of self-appraisal has been largely channelled through the Medical Society for the Study of Venereal Diseases and the work of the British Co-operative Clinical Group.

The present study was designed to collect information on diagnostic and reporting criteria used by clinics in England and Wales and to compare treatment and management policies. This was seen as a continuation of the tradition of self-appraisal and had two aims. Firstly, to allow individual clinicians to judge their own practices and standards against those of their peers, and secondly, to furnish information that might provide the basis for the

development of an improved and, possibly, a more rational service.

'In clinical work, as in other fields, unless information is available about the results of our actions, we do not have the option to make material adjustments to these actions in the future' (Acheson, 1968, unpublished).

Method

All consultant venereologists in charge of STD clinics in England and Wales were approached during the course of the study. A letter explaining the survey and inviting participation was followed by a telephone call to arrange an interview. Most consultants were interviewed personally by one of two doctors (MWA or BHO'C) using a standard questionnaire which had been tested in a pilot survey carried out on a 10% random sample of consultants. It was considered desirable to conduct the interview personally in view of the length and comprehensive nature of the questionnaires, but six were completed by post at the request of either the physicians or the research team. A postal interview was always followed by a telephone call to discuss any problems. Interviews lasted about an hour.

The questionnaire was designed to cover many aspects of organisation and clinical practice. Information was sought on facilities at the clinic and on the diagnostic, treatment, and reporting criteria for five specified diseases (Table 1). Since the amount of data obtained was substantial the study will be reported in a series of papers.

Results

NUMBER OF CONSULTANTS AND CLINICS

There are 103 consultants in charge of 189 clinics in England and Wales. Ninety-five agreed to take part, a response rate of 92% (Table 2). Eighty-nine (93%) of the interviews were carried out by personal interview and the remainder by post and telephone.

Most clinics were for men and women (Table 3). Of the single-sex clinics, three were held in an exclusively single-sex hospital—one for men and the other two for women.

Table 1 *Survey of sexually transmitted diseases clinics in England and Wales*

Aspects studied	
Clinic facilities	Hours open Contact tracing Microscopy Cultures Serology General diagnostic criteria
Diagnostic, treatment, and reporting criteria	Gonorrhoea Non-specific genital infection Trichomoniasis Herpes genitalis Candidiasis

Table 2 *Number of consultants and clinics in survey*

	Consultants	Clinics
Number in England and Wales	103	189
Number participating	95	175
Response rate (%)	92	92

Table 3 *Sex of the patients seen in clinics*

Patients	Clinics	
	No.	%
Men and women	169	96.6
Women	4	2.3
Men	2	1.1
Total	175	100

CLINIC HOURS

Clinics were open for between one and 49½ hours a week, the mean length of time being 14½ hours and the median 9½ hours. Figure 1 shows that 92 (53%) clinics were open for 10 hours or less a week and that 52 (30%) were open for five hours or less. Rarely was a clinic open all day for five days a week.

Most patients seen in clinics are in full-time employment and are unable to take repeated periods of time off work. It was therefore considered important to find out if clinics took this into account

by staying open after 5.0 p.m. and on Saturday mornings. Eighty-one per cent of clinics were open for some time during the week after 5.0 p.m. The period of time varied from half an hour to 10 hours a week. Only 2% of clinics remained open for 10 evening hours weekly, the mean period of time being three hours a week. Only 26 (15.0%) clinics were open on Saturday mornings.

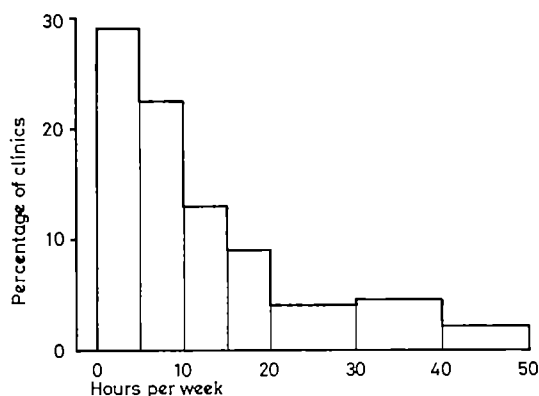


Fig. 1 *No. of hours clinics were open*

CLINICAL RESPONSIBILITIES

Most consultants worked in more than one clinic and some in as many as five (Table 4). Often the doctor had only nursing help. Two of the possible consequences of working in more than one clinic are that a doctor may not be present all the hours that the clinic is open and that, when present, he is unable to cope with the clinical load on his own. In these circumstances it may be necessary for the consultant to delegate some of the duties normally carried out by him to an appropriately trained nurse.

Table 4 *Number of clinics per consultant*

No. of clinics	Consultants working in clinics	
	No.	%
1	42	44.2
2	33	34.8
3	14	14.7
4	4	4.2
5	2	2.1
Total	95	100

In 85 (48%) clinics nurses were allowed to see and advise patients; in 24 (14%) clinics this was so if the doctor was absent (Figure 2). In such a case the nurse would tell the patient the times at which the doctor would be available and then advise the patient on the probable nature of symptoms and

any interim measures that should be taken. In 47 (27%) clinics nurses examined and diagnosed patients, in 17 (10%) clinics this occurred if a doctor was not at the clinic. If a doctor was absent the nurse would take the history, carry out the examination and relevant tests, and ask the patient to return for treatment when the doctor was available. In 35 (20%) clinics nurses were allowed to prescribe treatment, in 17 of them this

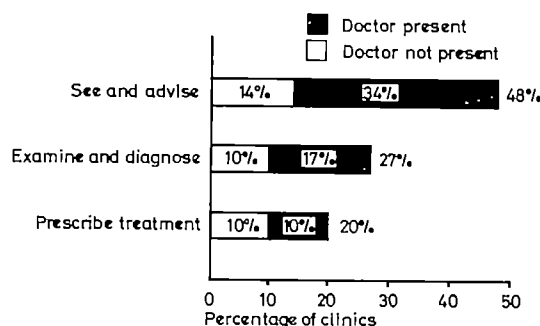


Fig. 2 Duties performed by nurses at clinics

was so when a doctor was not present. If the doctor was absent the nurse would examine and diagnose the patient and confer on the telephone with the doctor before instituting treatment. The clinics where nurses examined patients and made a diagnosis were open for a mean of 17 hours a week compared with a mean of 14½ hours for all clinics. The mean for clinics in which nurses prescribed treatment was 19 hours.

CONTACT TRACING

Table 5 shows that as many as 75% of clinics had a full or part-time contact-tracing service, and that only a few (20%) provided a clinical service without the support of contact tracers. Contact tracing was considered to be full-time if it was available during all hours that the clinic was open.

Table 5 Contact tracing in clinics

Contact tracing	Clinics	
	No.	%
Full-time	110	62.9
Part-time	22	12.6
None	35	20.0
Not known	8	4.5
Total	175	100

Table 6 shows the policy adopted for issuing contact slips for clinics with a contact-tracing service and for those without. There is little difference in the policy of giving slips between the two types of clinics. Information was not obtained about issuing contact slips to patients with syphilis.

MICROBIOLOGICAL, CULTURAL, AND SEROLOGICAL SERVICES

Microscopy

All the clinics had facilities for microscopical examination of Gram-stained slides for gonococci and *Candida albicans* and darkground examinations for *Treponema pallidum* and *Trichomonas vaginalis*. However, in seven (4%) of the clinics for men and nine (5%) of the clinics for women the smears had to be sent to a central laboratory and results were not available at the patients' initial attendance.

Cultures

Almost all the clinics (98.8%) had culture facilities (Table 7). The commonest procedure was to send the specimens to a central laboratory in a transport medium. In the 86 clinics that did this, 75 (87%) used Stuart's medium and 11 (13%) Amies' medium. The next most usual practice was for the staff to inoculate the plates in the clinic and send them to the laboratory. There was rarely a comprehensive laboratory service within the clinic.

Table 6 Diseases for which contact slips issued

Disease	Contact-tracing service			
	Clinics with (N=132)		Clinics without (N=35)	
	No.	%	No.	%
Gonorrhoea	126	95	33	94
Non-specific genital infection	83	63	23	66
Trichomoniasis	79	60	23	66
Herpes genitalis	53	40	16	46
Candidosis	49	37	14	40

Table 7 Culture facilities in clinics

Facility	Clinics	
	No.	%
Send in transport medium	86	49.1
Inoculate plates in clinic	78	44.6
Inoculate and read plates in clinic	9	5.1
None	2	1.2
Total	175	100

Serological tests for syphilis

The survey was not designed to collect detailed information about the diagnosis and treatment of syphilis, but there were questions about the facilities and types of serological tests carried out to exclude syphilis. All the consultants had a serological service available for their use and they were asked to indicate which tests they routinely requested at a patient's first visit, regardless of the possible diagnosis. Many laboratories were able to carry out

a fluorescent treponemal antibody absorption (FTA-ABS) test on specimens giving positive results even though this may not have been one of the initial tests. In most clinics more than one routine test was used; the combinations are shown in Table 8. In 11 (6%) clinics one test was used routinely, in 80 (46%) clinics two tests were used, and 84 (48%) clinics used three or more. The most

Table 8 *Combinations of routine serological tests performed in clinics*

Combinations of tests	Clinics	
	No.	%
VDRL	7	4.0
TPHA	2	1.1
WR	2	1.1
VDRL/TPHA	33	18.8
VDRL/FTA	4	2.9
VDRL/WR	9	5.2
VDRL/RPCFT	12	6.8
TPHA/FTA	2	1.1
TPHA/WR	1	0.6
TPHA/RPR	2	1.1
TPHA/RPCFT	1	0.6
FTA/RPCFT	1	0.6
WR/RPCFT	15	8.5
VDRL/TPHA/FTA	14	8.0
VDRL/TPHA/WR	3	1.7
VDRL/TPHA/RPR	1	0.6
VDRL/TPHA/RPCFT	5	2.6
VDRL/FTA/RPCFT	2	1.1
VDRL/WR/RPCFT	31	17.7
TPHA/WR/RPCFT	8	4.6
WR/RPR/RPCFT	2	1.1
VDRL/TPHA/WR/RPR	2	1.1
VDRL/TPHA/WR/RPCFT	9	5.1
VDRL/WR/RPR/RPCFT	2	1.1
VDRL/TPHA/FTA/WR/RPCFT	4	2.3
VDRL/TPHA/WR/RPR/RPCFT	1	0.6
Total	175	100

commonly used combination was the Venereal Disease Research Laboratory (VDRL) and the treponemal haemagglutination (TPHA) tests (33 (19%) clinics), followed by the VDRL with the Wassermann reaction (WR) and Reiter protein complement-fixation test (RPCFT) (31 (18%) clinics).

Although all clinics had a serological service available the doctors use of it varied (Table 9). In 10 (6%) clinics routine tests were performed only on selected patients for whom there were specific reasons. These reasons were the necessity to exclude syphilis if the patient had suggestive signs and symptoms, if sexual intercourse had taken place within the incubation period, or if the patient was homosexual or judged to be promiscuous.

More important than the failure of doctors in a few clinics to carry out initial serological tests was the number who did not later repeat the tests. In four (2%) clinics tests were never repeated and

Table 9 *Serological tests—policy of clinics*

Policy	First visit		Follow-up visit	
	No.	%	No.	%
Tests on all patients	165	94.3	79	45.1
Tests on selected patients	10	5.7	92	52.6
Tests never performed	—	—	4	2.3
Total	175	100	175	100

only 79 (45%) clinics of those performing serological tests would tests be repeated as a routine. In most clinics there had to be a specific reason for doing further serological tests at follow-up visits (Table 10). The most common reason was that the patient was a possible syphilis contact or had symptoms suggestive of this disease at the first consultation. In 53 clinics only one of the listed reasons was given for asking patients to return; 20 gave two and 19 clinics identified three or more possible reasons.

In the clinics which repeated serological tests on all or selected patients, 46 (27%) did the last test less than three months after the patient's history of initial exposure. However, in most clinics the last test was performed three months or later.

Table 10 *Reasons given by 92 clinics for repeating serological tests on selected patients*

Reason	Clinics	
	No.	%
Possible syphilis contact or symptoms of syphilis	46	50.0
Treated STD apart from syphilis	33	35.9
Sexual intercourse within last three months	10	10.9
Contact of other STD apart from syphilis	10	10.9
Homosexuality	20	21.7
Promiscuity	21	22.8
Other	13	14.1

SYMPTOMLESS PATIENTS

The papers that follow will report on clinical practice in relation to specific diseases. It is intended to describe here only the management of symptomless patients having sexual intercourse who presented for a check-up and who were not named contacts. Table 11 shows the sites from which samples were routinely collected in these patients. In most clinics samples were taken from the principal exposure sites. In passive homosexual patients this was the rectum and in women it was the urethra, cervix, and vagina. In heterosexual men the urethra was sampled routinely in only 58% of clinics and in active homosexual men in only 63% of clinics. However, in the clinics which did not take urethral specimens a two-glass urine test was routinely carried out.

Another feature of the assessment of symptomless patients was to find out whether investigations were

Table 11 *Sites from which samples routinely collected in symptomless patients presenting to clinics for check-ups*

<i>Patients</i>	<i>Sites</i>	<i>Clinics*</i>	
		<i>No.</i>	<i>%</i>
Men			
Heterosexuals	Urethra	99	57.9
	Rectum	108	63.1
Active homosexuals	Urethra	82	47.9
	Rectum	166	97.0
Passive homosexuals	Urethra	69	40.3
	Rectum	166	97.0
Women			
	Urethra	169	97.9
	Cervix	172	99.4
	Vagina	160	92.5

*171 clinics for men, 173 for women

repeated and, if so, on how many occasions if they had initially been negative. Table 12 shows that in most clinics attempts were made to see patients on at least a second occasion. Heterosexual men were less likely to be seen again. Of those clinics in which investigations were repeated, one-third did so only in selected instances—if the patient was promiscuous or homosexual, lived locally, or for reasons relating to the time interval between the last sexual intercourse and the initial tests.

Table 12 *Number of further occasions that tests were repeated in symptomless patients presenting for check-ups in whom initial tests were negative*

No. of times repeated	Clinics for men								Clinics for women					
	Heterosexuals				Homosexuals									
					Active		Passive							
	No.		%		No.		%				No.		%	
	No.	%	No.	%	No.	%	No.	%						
None	53	30.9	30	17.5	24	14.0	22	12.7						
1	66	38.6	59	34.5	58	33.9	65	37.6						
2	35	20.5	35	20.5	36	21.1	38	21.9						
3	7	4.1	31	18.1	37	21.6	36	20.8						
>3	8	4.7	14	8.2	14	8.2	10	5.8						
Not known	2	1.2	2	1.2	2	1.2	2	1.2						
Total	171	100	171	100	171	100	173	100						

QUARTERLY RETURNS

There are two steps in the completion of the quarterly returns for the DHSS; the first is making the definitive diagnosis from the notes and the second is the completion of the appropriate form (SBH 60). Table 13 shows which person was responsible for making the diagnosis and for supervising the completion of the returns. The senior doctor or director of the clinic was usually responsible for making the definitive diagnosis. However, the supervision of the completion of the returns was equally likely to be the doctor or the clerk/receptionist or the nurse/orderly/contact tracer.

Table 13 *Quarterly returns*

Individual responsible	Making the definitive diagnosis		Supervising the completion	
	No.	%	No.	%
Senior doctor or director	135	77.1	55	31.4
Junior doctor	5	2.8	1	0.6
Doctor seeing patient	8	4.6	0	0.0
Clerk or clinic receptionist	12	6.9	58	33.1
Nurse, orderly, or contact tracer	15	8.6	61	34.9
Total	175	100.0	175	100.0

Discussion

CLINIC HOURS AND SUPPORTING STAFF

Compared with other countries the service for sexually transmitted diseases in England and Wales is excellent. However, comparisons such as these may obscure the important issues facing the service in this country and generate inertia. It is salutary to note that the main problem facing the STD service today is exactly the same as that found when facilities for diagnosis and treatment were first established by the Venereal Disease Regulations of 1916—namely, a large increase in numbers of patients, a shortage of staff, and inadequate facilities. Catterall and Morton (1970) and Catterall (1973) have drawn attention to this and commented on the overcrowding in clinics, lack of new purpose-built facilities, long hours worked by consultants and other staff, and the difficulties in recruiting medical and ancillary staff.

The present study has revealed that half the clinics are open for 10 hours or less a week and that one-third are open for five hours or less. In 1968, 71% clinics were open for less than 10 hours a week (British Co-operative Clinical Group, 1971) and there has therefore been an improvement since then. Patients with STD should be seen as soon as possible after the development of symptoms. Anything that discourages this, such as having to wait several days before a clinic is open or having to travel long distances to another clinic, can make all the difference in controlling disease.

The supporting services are vitally important in the management of STDs. It has been pointed out that the nursing staff carry out many of the clinical and clerical duties within clinics. As long as the nurses are adequately trained this delegation would appear to be acceptable. The job of the clinical nurse has been reviewed recently by the DHSS and the Welsh Office. The circular shows an appreciation of the changing role of the nurse and encourages the delegation of work 'which has hitherto been carried out by doctors' but only after adequate and appropriate training (Department of Health and Social Security, 1977). The General Medical Council

(1977) has published revised guidelines on professional conduct and recognises the contribution that can be made to health care by nurses and other persons who have been trained to perform specialised functions.

Another important supporting service in the control of sexually transmitted diseases is contact tracing (Wigfield, 1972; Harris, 1975). The findings that 75% of clinics had the support of a full or part-time contact-tracing service is encouraging. In the remaining 20% of clinics it is hoped that doctors or nurses carry out this duty. Certainly most clinics (94%) without such a service did issue contact slips to patients with gonorrhoea. Dunlop (1963) studied the effectiveness of contact slips in controlling gonorrhoea and reported that this method was inadequate and advocated that specially trained contact tracers should be used. The absence of these trained staff in 20% of clinics in England and Wales places an additional workload on already busy physicians and ancillary staff. '... the interviewing of patients to obtain the necessary information and to persuade them to help in tracing their own contacts is very time-consuming and cannot be done satisfactorily by busy doctors, already hard pressed in the clinics' (*British Medical Journal*, 1974). Contact tracing is an integral part of the management of STDs and the lack of such a service may result in further spread of disease.

TRANSPORTATION OF CULTURES

It is encouraging that most clinics had a microscopical, cultural, and serological service. Half the clinics sent cultures to the laboratory in a transport medium and Stuart's medium was more often used than that of Amies' (Stuart, 1946; Amies and Garabedian, 1967). In most of the remainder culture plates were inoculated in the clinic and then transported to a central laboratory. In the study of the British Co-operative Clinical Group in 1968 transport media were used in 65% of clinics. At that time the consultants judged that they obtained poor or fair results in 29% of clinics using transport media compared with 19% of clinics using direct plating and transportation. This finding, plus the fact that some loss of organisms may be expected when material in transport medium is subsequently plated, probably accounts for the decline in the use of this method. However, the little scientific work that exists comparing the use of transport media with direct plating is contradictory. Danielsson and Johannisson (1973) compared results in specimens taken from men and women and placed in Stuart's transport medium and later plated on to both a selective Thayer Martin medium and a non-selective medium with specimens transported after direct

inoculation on to selective and non-selective media (Thayer and Martin, 1964). They found that the diagnosis of gonorrhoea would have been missed in 3% of women by using Stuart's transport medium with subsequent plating on to a selective medium, compared with direct plating on the same selective medium and then transporting the plates to the laboratory. Hosty *et al.* (1974) reported a larger loss: the use of Stuart's transport medium followed by plating on to Thayer Martin medium resulted in missing 44% of cases, compared with direct plating and transportation. However, they delayed plating from the transport to the definitive medium for 24 hours, which may account for some of this loss. Contrary evidence has been reported by Jephcott (1977) who, in a careful study in this country, showed that Stuart's transport medium was superior to transportation of inoculated plates. In view of this conflicting evidence no change in the current practice of performing and transporting cultures can be justified.

SEROLOGICAL TESTS

Several questions about serological tests for syphilis should be asked. These include—What should be the criteria for offering tests? What tests should these be and how often should they be performed? In 94% of clinics routine serological tests were performed on all new patients but in only 45% were the tests repeated as a routine. The most commonly stated reason for repeating a test was that the patient was a possible syphilis contact. Certainly this is a good reason, but as more than one STD is commonly diagnosed in the same patient it might be better to repeat serological tests on all treated patients. If this is not possible this might be done in all cases of treated gonorrhoea, non-specific genital infection, and genital ulceration not already diagnosed as syphilitic in origin.

The clinics used numerous serological tests to exclude syphilis. Consultants were asked to say which they used as a first line routine screening test. In 11 clinics only one test was used (VDRL seven clinics, TPHA two clinics, and WR two clinics). Of these tests the VDRL and WR used alone are of limited value since both are non-specific for anti-treponemal antibodies. The TPHA is a specific test, but on its own the titre will not help the clinician to differentiate untreated early syphilis from the patient with residual antibody (O'Neill, 1976). Also in the treated patient, the TPHA titre tends to fall extremely slowly which may cause confusion. Finally, if used on its own, untreated early syphilis can be missed. The TPHA is a cheap, good, and simple test, but it should be used in conjunction with another.

Physicians used a number of combinations of tests, not all of which are equally effective. The most common combination (31 (18%) clinics) was the VDRL test, WR, and RPCFT; in another nine (5%) clinics the VDRL and WR tests were used. None of these tests uses specific antigen and, therefore, has limited diagnostic value. In contrast, some clinics used more tests than were necessary. In 19 (11%) the VDRL test and TPHA were used routinely in conjunction with the RPCFT or with additional tests. The VDRL test and TPHA on their own are adequate screening tests and the addition of the RPCFT adds nothing to the clinician's understanding of the patient's disease, and adds greatly to the workload in the laboratory. A case could be made for reducing the number of tests performed on each sample of serum in such cases and ensuring that a second sample was taken three months after the first.

As stated above, the TPHA alone may miss the patient suffering from early primary syphilis and the same is true for the VDRL. This points to the importance of repeating serological tests at least three months after initial exposure. Unfortunately nearly one-third of clinics who repeated tests did so within three months.

SYMPTOMLESS PATIENTS

Patients who attend STD clinics requesting examination often give a history of recent casual sexual intercourse and are therefore at risk of contracting a sexually transmitted disease. In almost all the clinics rectal specimens were taken from symptomless passive homosexual patients and urethral and cervical specimens from women who asked for examination. This is probably because infection of these sites is less likely to present with overt symptoms than urethral infection. This may account for the fact that in the heterosexual males and active homosexuals who attend for a check-up, fewer consultants were prepared to take specimens from the urethral site. However, in view of the reports of asymptomatic urethral gonorrhoea and non-specific urethritis in men, this policy cannot be justified (Pariser *et al.*, 1964; Thatcher *et al.*, 1969; Rodin, 1971; Fox, 1974; Portnoy *et al.*, 1974; Handsfield *et al.*, 1974; Perera and Lim, 1975). In all the clinics in which urethral tests were not carried out a two-glass urine test was performed, but as Rodin (1971) pointed out, this test is not a reliable diagnostic test in many of these patients. Even if threads are found, the chance of obtaining a specimen for microscopy and culture is lost unless the patient returns, and Perera and Lim (1975) reported that 20% of their patients did not return.

Conclusions

This study has attempted to provide those working in the field of STDs with current information on facilities and clinical practice in England and Wales. It has been reported that clinics are open for more hours a week than 10 years ago and that most clinics have a full or part-time contact-tracing service. Each clinic had microscopical and serological services available and, almost all had cultural facilities. Half of the clinics used transport medium and this seems a justifiable approach in view of present knowledge. However, facilities were not always used in the most optimal way. In some clinics serological tests for syphilis were carried out singly, in inappropriate combinations, not repeated at all, or within three months of the patients' initial exposure. Finally, it has been suggested that urethral specimens might be taken from all asymptomatic male heterosexual and active homosexual patients who present to clinics for examination.

It is realised that many of the inadequacies and inconsistencies of the management of patients with STDs are organisational and outside the control of the physician. It is hoped that this and the papers that follow will provide the objective basis for those specialising in the management of STDs to discuss their own practices among themselves and with health service administrators with a resulting improvement in the service.

This study was supported as part of a project grant from the Medical Research Council. We should like to thank those who participated in the study and Dr C. S. Nicol and Dr R. S. Morton for their advice and comments at all stages of the study. We are particularly grateful to Dr R. R. Willcox for his generous advice on the design of the study.

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Diagnostic treatment and reporting criteria for gonorrhoea in sexually transmitted disease clinics in England and Wales

1: Diagnosis

M. W. ADLER

From the Departments of Venereology and Genitourinary Medicine and Community Medicine, Middlesex Hospital and Medical School, London

SUMMARY The current methods used in the diagnosis of gonorrhoea are reported. The sites sampled in symptomatic patients who were not gonorrhoea contacts and those sampled in symptomless contacts are described. Urethral specimens were taken routinely from all male heterosexual and active homosexual patients with a urethral discharge but in only 81% and 82% respectively of these patients if they were symptomless gonorrhoea contacts. Not all consultants would repeat investigations (smear and/or cultures) in gonorrhoea contacts if the initial ones were negative; heterosexual male and active homosexual patients were less likely to be seen again. Smears and cultures were performed routinely on women in 97% of clinics, but for men this was so in only 81% of clinics. The wider use of cultures for men, the policy with regard to the taking of urethral specimens from asymptomatic male heterosexual and active homosexual patients who are gonorrhoea contacts, and the number of diagnostic investigations required are discussed.

Introduction

Gonorrhoea is now the second most commonly treated sexually transmitted disease (STD) in England and Wales. It presents a number of problems, the foremost being that the number of notified cases has increased by 50% during the last decade, and that β -lactamase producing strains of gonococci have recently appeared (Ashford *et al.*, 1976; Percival *et al.*, 1976; Phillips, 1976).

The previous paper (Adler *et al.*, 1978) described the background and methods used in an inquiry into the present pattern of care for STDs in England and Wales. This paper is concerned particularly with the current management of gonorrhoea. The first part describes the diagnosis of gonorrhoea and the second the treatment and reporting criteria used by clinics.

Results

SITES SAMPLED

Symptomatic patients who are not contacts

Consultants were asked what methods they used to diagnose men or women presenting with symptoms suggestive of a STD who were not known to be gonorrhoea contacts. Table 1 shows the sites routinely sampled. In all clinics specimens were taken from the urethra in male heterosexuals and active homosexuals with genitourinary symptoms, and from the rectum in passive homosexuals. Specimens were taken routinely from the rectum in active homosexuals in only 96 (56%) clinics and urethral specimens from passive homosexuals in only 100 (58%) clinics. In the clinics in which specimens were not normally taken from these two sites this would be done if the patients admitted to being both active and passive or if they had additional signs or symptoms that warranted further investigations. In none of the clinics would a throat swab be taken in male heterosexual patients unless there were specific indications—such as a sore

Table 1 Sites sampled routinely in symptomatic patients (non-contacts)

Patients	Sites	Clinics	
		No.	%
Males (171)			
Heterosexuals	Urethra	171	100.0
	Throat	0	0.0
Active homosexuals	Urethra	171	100.0
	Rectum	96	56.1
	Throat	24	14.0
Passive homosexuals	Rectum	171	100.0
	Urethra	100	58.4
	Throat	24	14.0
Females (173)			
	Urethra	171	98.8
	Cervix	173	100.0
	Vagina	166	95.9
	Rectum	15	8.6
	Throat	3	1.7

throat or a history of oral sex. In the homosexuals, 24 (14%) clinics took throat specimens routinely even if these indications were not present. In almost all the clinics seeing women the urethra, cervix, and vagina were sampled (99%, 100%, and 96% respectively). The large number of clinics in which vaginal specimens were taken probably reflects the fact that the patients were symptomatic and not known to be gonorrhoea contacts, therefore making it necessary to exclude candidosis and trichomoniasis.

Symptomless patients who are contacts

The procedure adopted by physicians in relation to symptomless patients who presented for examination and who were not known to be contacts has already been reported (Adler *et al.*, 1978).

Consultants were also asked about their management of symptomless gonorrhoea contacts. The sites tested routinely on symptomless male contacts of patients with gonorrhoea are shown in Table 2. Urethral specimens were taken as a routine from symptomless heterosexuals in 138 (81%) clinics and

Table 2 Sites sampled routinely in symptomless male contacts of patients with gonorrhoea

Patients	Sites	Clinics (N=171)	
		No.	%
Heterosexuals	Urethra	138	80.7
	Prostate	15	8.7
	Throat	1	0.5
Active homosexuals	Urethra	140	81.8
	Rectum	93	54.3
	Throat	29	16.9
Passive homosexuals	Rectum	165	96.4
	Urethra	86	50.2
	Throat	29	16.9

in 140 (82%) clinics from active homosexuals. In 96% rectal specimens were taken from passive homosexual contacts. As with the symptomatic patients who were not contacts, fewer clinics (54%) took routine rectal specimens from active homosexuals and 50% took urethral specimens from passive homosexuals. However, if patients volunteered that either of these sites had been at risk or if they had relevant symptoms or signs, investigations were performed.

In 99% of clinics seeing symptomless female contacts of males with gonorrhoea specimens were taken as a routine from the urethra and all clinics took specimens from such patients from the cervix (Table 3). Vaginal specimens were taken in 91% of

Table 3 Sites sampled routinely in symptomless female contacts of patients with gonorrhoea

Sites	Clinics (N=173)	
	No.	%
Urethra	171	98.8
Cervix	173	100.0
Vagina	157	90.7
Rectum	35	20.2
Throat	9	5.2

clinics and once again this reflects the doctors' attempt to exclude candidosis and trichomoniasis in association with gonorrhoea. Rectal specimens were taken routinely in 35 (20%) clinics. Of the remaining 138 clinics in which routine rectal specimens were not taken, the consultants in 12 of them indicated that they were never taken, whereas in the other 126 clinics this was done if a specific reason existed. It was extremely rare for consultants to take routine throat specimens (nine clinics), in 16 clinics specimens were never taken from this site but in the remaining 148 they would be in certain circumstances. The reasons for which rectal and throat specimens were taken in those clinics, in cases in which it was not done routinely, are shown in Table 4.

Table 4 Reasons for taking rectal and throat specimens in female contacts of patients with gonorrhoea

Reason	Rectal specimens (N=126)		Throat specimens (N=148)	
	No.	%	No.	%
History of rectal/oral sex	35	27.8	44	29.7
Rectal/oral symptoms or signs	47	37.3	89	60.1
Gonorrhoea not found elsewhere	28	22.2	14	9.5
Gonorrhoea not found elsewhere plus rectal/oral symptoms or signs	16	12.7	1	0.7

Another facet of examining gonorrhoea contacts is their follow up if the initial investigations (smear and/or cultures) are negative. Table 5 shows the

Table 5 *Number of further occasions that examinations and/or tests repeated in gonorrhoea contacts in whom initial tests negative*

No. of times repeated	Hetero-sexuals		Active homo-sexuals		Passive homo-sexuals		Females	
	No.	%	No.	%	No.	%	No.	%
None	30	17.5	23	13.5	20	11.7	22	12.7
1	50	29.2	43	25.1	38	22.2	30	17.4
2	42	24.6	54	31.6	53	31.0	58	33.5
3	32	18.7	37	21.6	46	27.0	47	27.1
>3	17	9.9	14	8.2	14	8.1	16	9.3
Total	171	100.0	171	100.0	171	100.0	173	100.0

policy adopted. Heterosexual males and active homosexuals were less likely to be seen again, tests were not repeated on passive homosexuals in 20 (12%) clinics or on female patients in 22 (13%).

Investigations before treatment

Microbiological and culture services were available in most clinics, but they were not necessarily used to reach a diagnosis. Consultants were asked what methods of diagnosis they would apply in patients presenting with urethral, vaginal, or rectal discharge or symptoms (Table 6). Smears and cultures were

Table 6 *Methods of diagnosis used in symptomatic male and female patients*

Method	Clinics for men		Clinics for women	
	No.	%	No.	%
Smears and cultures in all cases	139	81.3	168	97.1
Smears in all cases and cultures in selected cases	24	14.0	3	1.7
History, examination plus smears only in all cases	8	4.7	2	1.2
Total	171	100	173	100

performed as a routine in nearly all the clinics (97%) seeing women but in clinics seeing men the physicians were more selective about the use of cultures. They were used routinely in all patients in only 139 (81%) clinics. The reasons for performing cultures in the 24 clinics for men, where it was done only on selected patients were as follows: equivocal smears (eight clinics), for possible legal reasons (seven clinics), patients at high risk (five clinics), and for rectal cases (four clinics).

In eight clinics where male patients were seen smears only were used even though a culture service

was available in seven of them. In the two clinics for women that used smears only, there was no option as these had no culture facilities.

Microscopical examination

All clinics had a microscopy service but in seven (4%) for men and in nine (5%) clinics for women the results were not available when patients first attended. The consultants were asked how they would manage such a situation in a symptomatic patient who was not a known contact (Table 7).

Table 7 *Action taken when results of smears from symptomatic patients (non-contacts) are not available at first consultation*

Action taken	Clinics for men		Clinics for women	
	No.	%	No.	%
Always treat	5	2.9	3	1.7
Selective treatment	2	1.2	3	1.7
Never treat	0	0.0	3	1.7
Not applicable (smears available)	164	95.9	164	94.9
Total	171	100	173	100

As the patient was not a contact for gonorrhoea or any other specific disease, and since smear findings were not available they would treat empirically if they so decided. Physicians in five out of the seven clinics for men that lacked immediate microscopy, indicated that they would treat the patient as suffering from gonorrhoea on the basis of the clinical history and examination. The consultants working in the two remaining clinics treated in selected instances—such as, if the patient was in discomfort or in transit. In the nine clinics for women without immediate smear results, three physicians would not institute treatment until after microscopical confirmation, three always treated for gonorrhoea on clinical evidence, and the remaining three only if the patient had suspected salpingitis or Bartholinitis, was in discomfort, or was about to leave the area.

Cultures

All the consultants working in clinics with a culture service, who had not already treated on clinical evidence, were prepared to treat male patients on the presumptive smear findings before the culture results were available. Most consultants would also treat women on these grounds but a few (8% of clinics with a culture service and where patients had not been treated on clinical evidence) would await culture confirmation. The reasons given were that the smear reading could be wrong, that there were legal reasons, and/or the woman was married.

It has been reported previously that 99% of clinics had access to culture facilities and that 49% of them used transport medium. The definitive type of media used for making or excluding the diagnosis of gonorrhoea are shown in Table 8. The most

tion was different in that in 32 (19%) of them smears alone were relied on in all or selected cases to establish a diagnosis in the symptomatic patient. In two recent books, the authors (Catterall, 1975; King and Nicol, 1975) suggest that microscopy should always be complemented by the use of cultures.

Osmond (1936) was among the first workers to show the necessity of using both techniques simultaneously in men. This has been confirmed in other more recent studies (Reznicek *et al.*, 1971; Dans and Judson, 1975). Dans and Judson (1975) compared urethral smears for gonorrhoea with culture results in men. They showed false positive results for smears ranging from 1.8% to 21% for different observers, the less experienced the observer the higher the rate. However, more important was the fact that the rate for smears considered to be negative which were subsequently positive on culture was between 1.4% and 8.3%. Reznicek *et al.* (1971) made similar observations and reported that in 11% of cases of gonorrhoea the smear was considered to be negative but the cultures were subsequently positive. These studies highlight the importance of using cultures to confirm microscopical findings and that skill and experience are required to prepare and read the slides.

It is interesting that these two studies (Reznicek *et al.*, 1971; Dans and Judson, 1975) are American as a pamphlet (Department of Health Education and Welfare, Public Health Service and Mental Health Administration, and Center for Disease Control, 1971) suggests that for males 'microscopic demonstration of Gram-negative intracellular diplococci on smear of urethral exudate constitutes sufficient basis for the diagnosis of gonorrhoea'. This view may be justified in situations in which resources and supporting staff are limited, but is the service sufficient if up to 11% of patients with gonorrhoea may be missed because of the lack of a culture service or failure to use it? The British Co-operative Clinical Group (1971) reported that smears and cultures were used in all men in 59% of clinics. The present study shows that there has been a change since that time and that now 81% of clinics do this. No doubt this improvement will continue. Notwithstanding this increased use of cultures it is important that microscopy should be as accurate as possible. The staining and examination of smears calls for skill and experience. It is important that doctors and nurses are adequately trained in these techniques and that any disparity between smears and cultures is continually monitored. This can be done by retaining smears until the culture reports are available and by re-examining the slides if there is any difference, thus allowing for regular evaluation.

Table 8 Culture media used to exclude or confirm diagnosis of gonorrhoea

Culture medium	Clinic (N=173*)	
	No.	%
1. Chocolate agar with antibiotic (Thayer-Martin or modifications)	72	41.6
2. Chocolate agar without antibiotic	25	14.6
3. Columbia blood agar with antibiotic	13	7.5
4. Columbia blood agar without antibiotic	11	6.3
5. Gonococcal selective	8	4.6
6. Difco selective	8	4.6
7. Transgrow	3	1.7
8. Others	5	2.9
1+2	6	3.5
1+3	4	2.3
2+4	5	2.9
2+5	1	0.6
3+4	8	4.6
3+8	1	0.6
Not known	3	1.7
Total	173	100

*2 Clinics—no culture service available

commonly used medium was a Thayer Martin medium or one of its modifications. In some clinics more than one medium was used equally often and the names of these are given. In 80 (46%) clinics sugar fermentation was carried out to verify that the Gram-negative organism found was *Neisseria gonorrhoeae*. In a further 29 (17%) clinics fluorescent techniques were used in addition to sugar fermentation and in 12 (7%) clinics fluorescence was used on its own. In 51 (29%) clinics neither of these two supportive tests was performed.

Discussion

THE USE OF CULTURES

The fact that in almost all the clinics (97%) smears and cultures were performed in symptomatic women is a reflection and appreciation of the difficulties in excluding the diagnosis of gonorrhoea in the female on microscopical evidence alone. Many authors have reported on the poor correlation between smear and culture results (Macleod *et al.*, 1934; Cooper *et al.*, 1950; Catterall, 1970; Frisch, 1975; Barlow *et al.*, 1976; Evans, 1976). Even some of the more contemporary work suggests that 45% of cases confirmed subsequently on culture would be missed by the use of smear alone. In clinics for men the situa-

SITES SAMPLED/REPEATING DIAGNOSTIC TESTS

Once a patient presents at a clinic the diagnosis of gonorrhoea should not be missed. The previous paper was concerned with the problems of asymptomatic disease in relationship to symptomless patients requesting examination. It was reported that in only 58% of clinics would a urethral smear be taken from the heterosexual male and in 63% of clinics from the active homosexual. When the patient is a named gonorrhoea contact the proportions are 81% and 82% respectively. This is an improvement but if there is a history of exposure, in the light of our current awareness of asymptomatic gonorrhoea, it is recommended that all male heterosexuals and active homosexuals should have urethral tests taken.

It has been pointed out that there are difficulties in making a firm diagnosis of gonorrhoea in women using Gram-stained smears, which is all that is available at the time of the first visit. It is therefore desirable that women be encouraged to return for a second visit by which time the culture results are usually available. Even so, recent work suggests that the culture results are not infallible. Catterall (1970) drew attention to this and showed that if only one set of investigations was performed 34% of cases would be missed, after two sets it would be 14%. Later work, using Columbia blood agar as a medium instead of the previous McLeod's chocolate agar, showed an improvement but one set of investigations was still negative in 9% of patients, and 2% were missed after two sets (Chipperfield and Catterall, 1976). These figures have been confirmed by others (Barlow *et al.*, 1976; Evans, 1976). In view of these reports it is important that all female contacts of gonorrhoea should have cultures repeated at least once, and preferably twice, if the initial smears and cultures give negative results. This was not universal practice in the clinics studied. In 13% of clinics seeing female patients investigations were not repeated if the initial ones were negative. Likewise female contacts need not be examined more than twice after the original test (three tests in all). Tests were repeated three or more times on female patients who were gonorrhoea contacts in 36% of clinics.

Conclusions

It has been reported that almost all consultants seeing symptomatic women perform smears and cultures as a routine. For men there has been a substantial improvement since the last British

Co-operative Clinical Group (1971) study, but cultures are not used routinely in 19% of clinics. It is suggested that the use of cultures allows for evaluation of microscopical techniques within clinics and that this should be a continuous process. Likewise not all consultants would take urethral specimens from asymptomatic heterosexual male and active homosexual patients who were gonorrhoea contacts. Physicians should be encouraged to take specimens from this site in these patients. Finally it is suggested that a more rational approach be adopted in repeating tests in women who are gonorrhoea contacts in whom the initial sets of tests are negative.

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Diagnostic treatment and reporting criteria for gonorrhoea in sexually transmitted disease clinics in England and Wales

2: Treatment and reporting criteria

M. W. ADLER

From the Departments of Venereology and Genitourinary Medicine and Community Medicine, Middlesex Hospital and Medical School, London

SUMMARY The current methods of treatment and reporting are described. The most common form of treatment for gonorrhoea in men and women was a single dose of oral ampicillin, usually 2 g. In proportionately more of the clinics treating women, treatment was given over several days. The wide use of ampicillin represents a marked change in treatment practice during the last decade. Procaine penicillin was the most commonly used parenteral preparation. The two dosages used most often for men were 1.2 and 2.4 megaunits. In women the commonest regimen was 2.4 megaunits and again there was a tendency for treatment to be given over several days. Epidemiological treatment was used widely and in one-third of clinics this occurred without confirmation that the patient was a true contact. Consultants used varying diagnoses on the quarterly returns for patients treated epidemiologically in whom the smears and/or cultures were negative. Most clinics classified these cases as 'other conditions requiring treatment' (D2) but as many as 19% of clinics designated these cases as being 'true' gonorrhoea. It is suggested that this results in an overestimate of the number of cases of 'real' gonorrhoea treated in England and Wales.

Introduction

Part 1 of this paper (Adler, 1978) was concerned with the current methods of diagnosis of gonorrhoea used by consultants in England and Wales. This part describes treatment and reporting criteria.

Results

TREATMENT FOR GONORRHOEA

Curative treatment with antibiotics

Tables 1 and 2 show the various types of treatment used for men and women diagnosed as suffering from gonorrhoea. For both sexes oral and parenteral preparations were used in equal proportions. Penicillin in one of its various forms was used most often.

For men the most commonly prescribed drug was ampicillin, being used in 58 (34%) clinics. In nearly all the clinics (54) a single dose ranging from 0.5 to 3.5 g was given, the commonest dose was 2 g (26 clinics) followed by 1 g (19 clinics) (Figure). In 53 of the 54 clinics using a single dose of ampicillin this was combined with 1 g of probenecid. In four clinics more than one dose was given, ranging from 1 g a day for three days to 0.5 g four times a day for six days. Three of these clinics also used probenecid.

Procaine penicillin was the most commonly used parenteral preparation. Of the 36 (21%) clinics for men in which it was used, 30 gave a single dose ranging from 1.2 to 2.4 megaunits. The two dosages most often used were 1.2 megaunits (11 clinics) and 2.4 megaunits (11 clinics). Probenecid was used less often by clinics in which procaine penicillin was prescribed than by those using ampicillin; with ampicillin it was 98% (53 out of 54) but with procaine penicillin 50% (15 out of 30). It is also interesting to note that probenecid was used more

Address for reprints: M. W. Adler, Medical Research Council, Sexually Transmitted Diseases Project, James Pringle House, 73-75 Charlotte Street, London W1N 8AA

Received for publication 8 September 1977

Table 1 *Drugs used to treat men with gonorrhoea*

Drug	Doseage		
	Once only	More than once	
Oral preparations			
Ampicillin			1 g, 3/7-0.5 four times a day, 6/7 (4)
Co-trimoxazole	0.5-3.5 g (54)		2 twice daily, 2/7-2 twice daily, 8/7 (14)
Tetracycline	8 tablets (2)		0.25 g four times a day, 5/7
	—		5/7-0.5 g four times a day, 5/7
Triple tetracycline (Deteclo)	2-2.5 g		1.2 g twice daily, 1/7-300 mg twice daily, 5/7
Amoxycillin	—		250 mg four times a day, 5/7
Tetracycline hydrochloride + nystatin (Mystectin)			
Parenteral preparations			
Procaine penicillin	1.2-2.4 megaunits (30)		1.2 megaunits, 2/7-3 megaunits, 3/7 (6)
Fortified procaine penicillin	1.2-2 megaunits		—
Benethamine penicillin, procaine penicillin, and benzylpenicillin (Triplopen)	1.2-2.5 megaunits (16)		—
Benzylpenicillin	5 megaunits		1.2 megaunits, 3/7 (4)
Spectinomycin	2 megaunits		—
Combinations			
Procaine penicillin + ampicillin	1.2 megaunits + 1 g		—
Procaine penicillin + amoxycillin	1.2 megaunits + 1 g		—
Procaine penicillin + triple tetracycline	1.8 megaunits		300 mg three times a day, 4/7
Procaine penicillin + tetracycline	0.6 megaunits		250 mg four times a day, 1/52
Benethamine penicillin, procaine penicillin + benzylpenicillin + co-trimoxazole	3-1 megaunits		2 twice daily, 6/7
Ampicillin + triple tetracycline	2 g		300 mg twice daily, 5/7

Table 2 *Drugs used to treat women with gonorrhoea*

Drug	Doseage		
	Once only	More than once	
Oral preparations			
Ampicillin			1 g twice daily, 1/7-0.5 g four times a day, 5/7 (10)
Co-trimoxazole	0.5-3.5 g (45)		2 twice daily, 2/7-2 twice daily, 8/7 (14)
Tetracycline	8 tablets (2)		0.25-0.5 g four times a day, 5/7
Triple tetracycline (Deteclo)	—		1.2 g twice daily, 1/7-300 mg twice daily, 5/7
Amoxycillin	2-2.5 g		—
Tetracycline hydrochloride + nystatin (Mystectin)	—		250 mg four times a day, 5/7
Parenteral preparations			
Procaine penicillin	1.2-3 megaunits (30)		1.2 megaunits, 2/7-3.0 megaunits, 3/7 (9)
Fortified procaine penicillin	2 megaunits		—
Benethamine penicillin, procaine penicillin, and benzylpenicillin (Triplopen)	1.2-2.5 megaunits (16)		1.2 megaunits, 3/7 (4)
Benzylpenicillin	5 megaunits		—
Spectinomycin	2-4 megaunits		—
Combinations			
Procaine penicillin + ampicillin	1.2-3 megaunits + 1 g (6)		500 mg four times a day, 3/7-4/7 (2)
Procaine penicillin + tetracycline	0.6 megaunits		250 mg four times a day, 1/52
Procaine penicillin + amoxycillin	1.2 megaunits + 1 g		—
Benethamine penicillin, procaine penicillin and benzylpenicillin + co-trimoxazole	3-1 megaunits		2 twice daily, 6/7

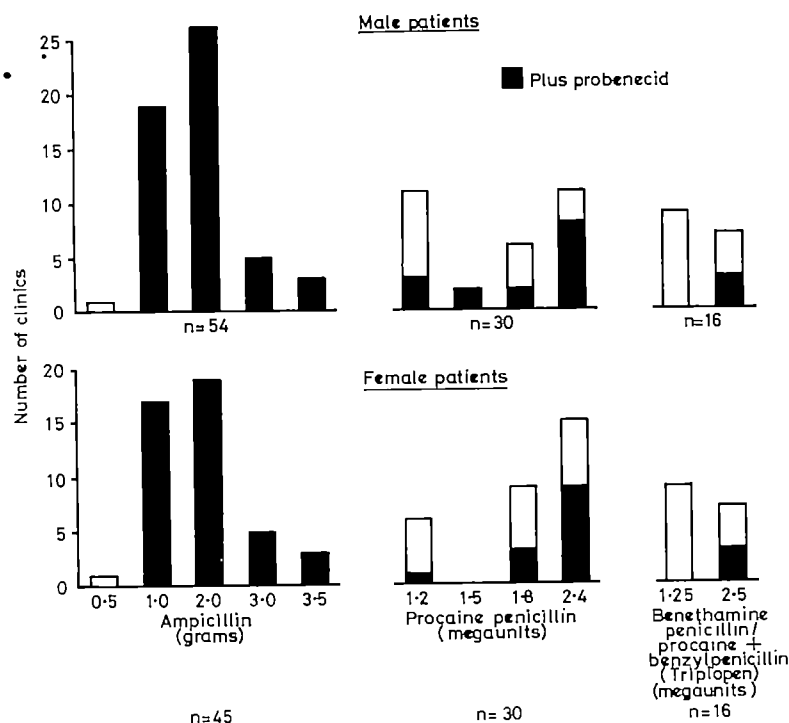


Figure Distribution of dosages for the three most commonly used drugs in the treatment of gonorrhoea (once only dosages).

often with higher doses of procaine penicillin than with lower. In six clinics procaine penicillin was used more than once, with doses ranging from 1.2 megaunits daily for two days to 3 megaunits daily for three days. Only one of these clinics used probenecid. Benethamine penicillin, procaine penicillin, and benzylpenicillin (Triptopen) was the parenteral preparation next most often used, being prescribed in 20 (12%) clinics for men. It was usually given as a single dose of 1.25 megaunits (9 clinics) but it was never combined with probenecid. The higher dosage of 2.5 megaunits was used in seven clinics and at this dosage it was more likely to be combined with probenecid. This trend of giving probenecid with higher doses is similar to that found with procaine penicillin.

The pattern of treatment for women was similar, with ampicillin being the most commonly used treatment followed by procaine penicillin. As with men the single dosage of ampicillin normally used was 2 g (19 clinics), the next most common was 1 g (17 clinics). However, in proportionately more of the clinics for women treatment was given over several days—10 (18%) out of 55 compared with

clinics for men—4 (7%) out of 58. The pattern was also true in relation to treatment with procaine penicillin, but to a lesser extent. The single dose of procaine penicillin most often given was 2.4 megaunits (15 clinics) followed by 1.8 megaunits (9 clinics). Again it was commoner to use higher dosages for women than men. The use of probenecid was similar to that in clinics for men in that it was used in all but one clinic prescribing ampicillin as a single dose, and more often with higher doses of benethamine penicillin, procaine penicillin, and benzylpenicillin than with lower ones.

Epidemiological treatment

In the present study, epidemiological treatment was defined as treatment given to named contacts after a history of exposure but without or in advance of confirmatory pathological findings—namely, treatment in advance of diagnosis. Table 3 shows the proportion of clinics in which this approach was used for different types of patients. Consultants were less inclined to use it for the active homosexual and male heterosexual patients, but more likely to use it for women. Many physicians were prepared

Table 3 *Number of clinics in which consultants were prepared to use epidemiological treatment*

Types of patient	Clinics	
	No.	%
Women	148	85.5
Homosexuals		
Passive	85	50.0
Active	64	37.6
Heterosexual men	71	41.5

to use epidemiological treatment, but in only one-third of clinics was it used in most cases; in the remainder it was used in selected patients. Those clinics in which this approach was used in most instances were open for a mean of 13 hours a week, and those that used it only in selected cases were open for a mean of 15 hours (mean for England and Wales = 14½ hours). The commonest reason for using epidemiological treatment was uncertainty that the contact would return to the clinic for results of the tests or to have further tests carried out (Table 4). In one-third of clinics one of the specified reasons was given, in one-third two were given, and in the final third three or more.

Table 4 *Reasons for the use of epidemiological treatment in selected gonorrhoea contacts*

Reason	Clinics (N=100)	
	No.	%
Fear of patient defaulting	60	60.0
Patient in transit	56	56.0
Pregnancy	29	29.0
Promiscuity/prostitution	27	27.0
Extramarital sexual intercourse	15	15.0
Reinfection/relapse of original patient	10	10.0
Possible damage to stable relationship	7	7.0
Women with possible salpingitis	4	4.0
Women menstruating	2	2.0
Anxious contact	2	2.0
Recent antibiotics	1	1.0
Patient institutionalised	1	1.0

In one-third of all the clinics using epidemiological treatment a verbal history of contact was considered an adequate reason for instituting epidemiological treatment; these clinics did not demand a contact slip or seek confirmation from another clinic. In the remaining two-thirds of clinics criteria were more stringent and either a slip or confirmation was required.

It was assumed that the consultants who used epidemiological treatment would examine patients and give tests before instituting therapy. However, this was not always so and in three clinics the physicians did not see the contact and supplied the patient with an extra course of treatment to

give to him or her. This procedure cannot be described as epidemiological treatment but constitutes treatment by proxy.

A further important aspect of epidemiological treatment is that concerned with the definitive diagnosis for the quarterly returns. Naturally if the tests for gonorrhoea are returned as positive, the patient is automatically counted as a case. However, it was also considered of interest to establish the eventual diagnosis used when a patient had been treated epidemiologically but the tests were negative. Table 5 shows the diagnostic categories used by clinics when the smears and/or cultures were negative and the patient had already been treated. The diagnostic category most often used was that of 'other conditions requiring treatment' (D2). However, in 19% of clinics consultants included these cases in the quarterly returns as if they were proved cases of gonorrhoea.

Table 5 *Diagnostic category used for contacts treated epidemiologically in whom smears and/or cultures were negative*

Diagnostic category	Clinics (N=148)	
	No.	%
Other conditions requiring treatment (D2)	106	71.6
Gonorrhoea (B1)	28	18.9
Other conditions not requiring treatment (D3)	7	4.7
Non-specific genital infection (C4)	7	4.7
No return	1	0.6
Total	148	100

Empirical/clinical treatment

This is treatment given to symptomatic patients, who are not named contacts, in advance of smears and/or culture confirmation. It was reported in part 1 of this paper that in some clinics, although a microscopy service was available, treatment was instituted on clinical evidence because smear results were not available at the patient's first attendance. In these circumstances the physicians tended to treat symptomatic patients as if they had gonorrhoea, even when they were not gonorrhoea contacts. In seven clinics for men treatment was based on the history and clinical findings. In four clinics the doctors used one form or another of penicillin and in the remaining three either co-trimoxazole or tetracycline was used. In six clinics women were treated according to the history and clinical findings, in three clinics penicillin was used, and in the other three co-trimoxazole or tetracycline was prescribed.

Prophylactic treatment

For the purpose of the study prophylactic treatment was defined as treatment given to a symptomless

patient before or after exposure but without knowledge of the state of health of the consort. This was used in selected cases in 29 (17%) clinics for men, and in 22 (13%) clinics for women (Table 6). No consultant using this technique counted these as cases of gonorrhoea, but most classified them as 'other conditions requiring treatment'. In 93% of clinics for men and in 77% of clinics for women using this approach penicillin was prescribed in the form of ampicillin, procaine penicillin, or benethamine penicillin, procaine penicillin, and benzylpenicillin (Triplopen).

Table 6 *Reasons for the use of prophylactic treatment*

Reason	Clinics for men (N=29)		Clinics for women (N=22)	
	No.	%	No.	%
Extramarital sexual intercourse	22	75.9	10	45.5
Going or returning from abroad	3	10.3	4	18.2
Patient insists	3	10.3	0	0.0
Medical colleagues	1	3.5	0	0.0
Irresponsible/low IQ	0	0.0	5	22.7
Other	0	0.0	3	13.6
	29	100	22	100

ALCOHOL

Most consultants asked patients to refrain from taking alcohol during treatment for gonorrhoea. This procedure was adopted in 152 (89%) clinics for men and in 132 (76%) clinics for women. The stated reasons for doing so varied (Table 7). The commonest reason was that alcohol irritates the urethra and increases the severity of the symptoms. This is quite different from having a relapse or a post-gonococcal urethritis which was another commonly cited reason. The second commonest reason was that physicians felt abstinence from alcohol would lessen the chances of sexual intercourse resulting from the patient's intoxication. Some doctors admitted that they advocated cessation of alcohol since it was standard and historical practice but were not sure that it was of any use. In two-thirds of clinics one of the reasons was specified and in the remaining third, two of them.

Table 7 *Reasons for refraining from alcohol during treatment for gonorrhoea*

Reason	Clinics (N=156)	
	No.	%
Irritates the urethra	75	48.1
Patients should remain sober	44	28.2
Relapse/post-gonococcal urethritis	41	26.3
Standard practice	25	16.0
Delays drug absorption	19	12.2
Other	16	10.3

FOLLOW-UP TESTS AFTER TREATMENT

All patients were seen on at least one further occasion for follow-up tests of cure after treatment (Table 8). The only exception to this was one clinic where male heterosexual patients were never seen again. In most clinics patients were seen on three or more occasions for repeat investigations, other than serological tests.

Table 8 *Number of further occasions that patients with gonorrhoea seen and tests repeated after treatment*

No. of times	Heterosexuals		Homosexuals		Women	
	No.	%	No.	%	No.	%
None	1	0.6	0	0.0	0	0.0
1	17	9.9	16	9.3	16	9.3
2	38	22.2	35	20.5	29	16.9
3	55	32.2	72	42.1	77	45.1
>3	60	35.1	48	28.1	49	28.7
Total	171	100	171	100	173	100

QUARTERLY RETURNS FOR GONORRHOEA

Information was obtained about the criteria used in making a return for gonorrhoea to the Department of Health and Social Security (DHSS). Consultants were asked to specify the types of cases and tests required for diagnosing a case of gonorrhoea on the quarterly returns (Table 9). Virtually all the physicians who had microscopical and cultural facilities, and used them, would notify a case if either test was positive.

It was reported (page 13) that in 32 clinics for men and in five for women the diagnosis was made from smears in all or selected patients. In 31 of these clinics the positive smear on its own was considered adequate to identify a case of gonorrhoea, this represents 18% of all clinics in England and Wales. In seven clinics treatment was given in all or selected instances on empirical or clinical grounds.

Table 9 *Criteria used in making a return for gonorrhoea to the Department of Health and Social Security*

Types of cases	Clinics (N=175*)	
	No.	%
With positive smears and positive cultures	173	98.8
With positive smears and negative cultures	170	97.1
With negative smears and positive cultures	172	98.2
With positive smears only (no culture service or not used)	31	17.7
Treated on clinical evidence (no microscopy or cultures)	4	2.3
Treated epidemiologically	28	16.0
Treated prophylactically	0	0.0

*2 clinics no culture service available

In four of the clinics a return would be made to the DHSS from the clinical diagnosis without supportive tests.

Discussion

ANTIBIOTICS

Consultants prescribing treatment for men and women used 17 and 15 respectively different routine treatment schedules for gonorrhoea. Penicillin in one of its various forms was the commonest type of treatment used, the most popular being oral ampicillin followed by parenteral procaine penicillin.

When ampicillin was first produced in 1961 it was found to have qualities that would allow it to be used in the treatment of gonorrhoea and as a possible alternative to the then widely used parenteral preparations of penicillin. Pharmacologically it has been shown to have distinct advantages over previous oral preparations in that it is acid stable, undergoes less protein binding, and gives a high blood concentration greatest within one to three hours (Acred *et al.*, 1963; Kunin, 1966). In addition it is faster and cheaper to administer, less painful to the patient, with little risk of anaphylaxis and sensitisation.

Only two years after the introduction of ampicillin Willcox (1963) was justified in saying that 'oral antibiotic therapy for the treatment of gonorrhoea... has not so far had the approval of most venereologists'. In 1968 this was confirmed by the study that he carried out for the British Co-operative Clinical Group (1971) into gonorrhoea practices in Great Britain. This study showed that in those clinics prescribing penicillin, procaine penicillin was used particularly often, being given in 57% of clinics for men and 54% of clinics for women. No clinic used ampicillin on its own. The present study indicates that, less than a decade after this last survey, there has been a marked change in treatment practices. Ampicillin is now the single most common form of treatment, being used in one-third of clinics.

This change is partly because treatment with ampicillin is more acceptable to patients and easier to administer in busy clinics than parenteral preparations. The other important factor is the efficacy of the treatment and it has taken a number of years to establish this. Studies were carried out initially to clarify the most appropriate dosage of ampicillin in the treatment of gonorrhoea (Alergant, 1963; Willcox, 1964). These studies used single doses of ampicillin but gave conflicting results, Alergant (1963) reporting a low failure rate (5%) and Willcox a higher one of 15%. This prompted Willcox (1965) to examine whether the drug needed to be given more than once. He showed that ampicillin given

as two doses of 1 g divided by five hours gave better results than a once only dose of 1 g, a finding confirmed by others (Groth and Hallqvist, 1970). The use of a divided dose raised doubts about patient compliance and prompted other workers to add probenecid to the regimen so that it could be given only once. Gundersen *et al.* (1969) was the first person to tackle this problem and showed that a combination of 2 g of ampicillin plus 1 g of probenecid given once gave a failure rate of only 1.2%. The final step in the acceptance of ampicillin was in studies that showed that 2 g of ampicillin plus probenecid was as effective as 1.2 megaunits of procaine penicillin plus probenecid (Cobbold *et al.*, 1973; Willcox *et al.*, 1973). Many of these series are not comparable as different follow-up intervals are used after treatment, criteria of treatment failure vary, and information on penicillin sensitivities of the gonococci is not always available.

It is now accepted that if ampicillin is to be used in the treatment of gonorrhoea the most appropriate dosage is a single one of 2 g combined with probenecid. In the present study this was the most commonly used dosage but many clinics were using the lower dosage of 1 g of ampicillin.

The next most common form of treatment was procaine penicillin. In clinics treating men doses of 1.2 and 2.4 megaunits were used equally often, whereas in the clinics treating women the higher dosage was more often used. The fact that clinics treating women tended to use higher dosages is probably a reflection of the difficulty in establishing proof of cure and the concern that they may develop complications—particularly salpingitis. This also accounts for the fact that doctors treating women gave both procaine penicillin and ampicillin in more than one dose more often than for men.

Curtis and Wilkinson (1958) reported that the use of long acting penicillin preparations could result in resistant strains of gonococci and asymptomatic carriers. Both of these situations now exist and even though they are not attributable to the use of any particular antibiotic it is surprising that a preparation such as benethamine penicillin, procaine penicillin, and benzylpenicillin (Triptopen) is still being used so widely. After procaine penicillin it was the most common parenteral preparation of penicillin, being used in 12% of clinics for men and in 11% of clinics for women.

Probenecid was used extensively with ampicillin but less often with procaine penicillin or benethamine penicillin, procaine penicillin, and benzylpenicillin (Triptopen) especially for the smaller dosages. Cobbold *et al.* (1970) and Rodin and Seth (1972) have reported substantially lower failure rates after treatment with 1.2 megaunits of procaine peni-

cillin combined with probenecid than when the same dose is given without probenecid. A dose of 1.2 megaunits of procaine penicillin is a perfectly acceptable level of treatment, but, in view of this work, it should be combined with probenecid much more readily than when using higher dosages when a certain loss of penicillin through renal excretion is less likely to affect the chances of eventual cure.

Co-trimoxazole was the treatment of choice in 9% of clinics, almost all the consultants prescribing treatment over several days. Even though co-trimoxazole has been shown to be effective in the treatment of gonorrhoea (Csonka and Knight 1967; Arya *et al.*, 1970; Carroll and Nicol, 1970; Rodin and Seth, 1972), there still remains the problem of compliance. Tablets that have to be taken outside the clinic for a number of days are more likely to be thrown away, stopped before the full course is finished, shared with one or more contacts, or used at a later date in an attempt at self-treatment.

Tetracycline or triple tetracycline (Deteclo) was used in eight clinics for men and in nine clinics for women. Although reasonable results have been obtained using tetracycline or triple tetracycline in the treatment of gonorrhoea (Smithhurst, 1970; Willcox, 1971) compliance is still a problem. In addition there are concerns in relation to sensitive strains of gonococci and cross resistance 'Because of the rapidity with which less sensitive strains of gonococci develop, and the increasing numbers showing cross resistance it is probably advisable not to use tetracyclines for gonorrhoea at the present time' (*Lancet*, 1972).

EPIDEMIOLOGICAL TREATMENT

Although epidemiological treatment was used widely throughout England and Wales, the study was not designed to collect opinions about the efficacy of this approach. This has been debated without resolution by venereologists for a number of years (Willcox, 1954, 1968, 1973; King, 1954, 1968; Brown, 1973; King and Nicol, 1975). It seems probable that epidemiological treatment will continue to be used by physicians, and this being so, the present survey has highlighted a number of areas for concern. In view of the lack of precision of this form of treatment it is essential that the doctor be certain that the patient is a true contact at risk of infection. Consultants working in as many as one-third of clinics prescribing epidemiological treatment did so on the consort's verbal history alone, without the production of a contact slip and without confirming with the other clinic what the diagnosis or treatment had been. Patients are often confused or too overwhelmed to remember their diagnosis or treatment and are therefore unlikely

to be able to give exact details to their contacts. Ten years ago a history of treatment by injection would have helped the doctor to establish the most likely diagnosis. But, as has been shown, gonorrhoea is now often treated by tablets and capsules which makes it difficult to differentiate the diagnosis from the more common disease of non-specific genital infection. It is suggested that, before the institution of epidemiological treatment, clinics should be certain of the disease to which the contact has been exposed. This can be achieved only by requiring the production of a contact slip or telephoning the clinic at which the patient was treated.

After epidemiological treatment has been used and the cultures have confirmed the diagnosis, it is perfectly reasonable to count the case as gonorrhoea for the purpose of the quarterly returns. However, if the cultures are negative it is then unjustifiable to classify the patient as having gonorrhoea. In these circumstances most clinics using epidemiological treatment (72%) designated their case as 'other conditions requiring treatment' (D2), but as many as 19% of clinics classified the case as being one of gonorrhoea. This leads to an overestimate of the true number of cases of gonorrhoea treated in England and Wales. The present study did not attempt to calculate the amount of inflation resulting from this practice but further work is being planned to estimate this.

EMPIRICAL/CLINICAL TREATMENT

In seven clinics for men and in nine for women smears were not available at the first consultation. Consultants in all the clinics for men and in six of the clinics for women said that they would always treat on the history and clinical findings, or in selected instances. In these circumstances most of the consultants treated the patient as if he had gonorrhoea by giving ampicillin or procaine penicillin. Although some discharges in women strongly suggest a diagnosis, macroscopic evidence is not suitable for an accurate diagnosis; this is especially so in men. If the patient is suffering from non-specific urethritis the use of penicillin is unlikely to give a cure. It is suggested that consultants seeing symptomatic patients, who are not gonorrhoea contacts, should wait for the results of smears and/or cultures so that they can select the most appropriate treatment. Understandably the physician faced with a patient complaining of a discharge will wish to offer immediate treatment, but he should realise that he may be choosing an inappropriate treatment which will be of little long-term benefit to the patient. The solution to this problem is for the consultant without immediate microscopy to insist

P 25,894

that a microscope is essential for making an immediate presumptive diagnosis.

PROPHYLACTIC TREATMENT

Prophylactic treatment was used in selected cases in 29 clinics for men and in 22 for women. Whereas there are reasonable arguments for using epidemiological treatment in selected cases it is hard to find any for prophylactic therapy. It is impossible for the clinician to guess whether or not a patient will contract or has contracted an STD while abroad or having casual sexual intercourse. More importantly it is impossible to select an appropriate form of treatment if one has no idea what disease or diseases one is supposed to be treating. Clinics favoured a form of penicillin when treating prophylactically. This therapy is really only effective against gonorrhoea, it can mask syphilis or inadequately treat it, and encourage the spread of penicillin-resistant organisms.

FOLLOW-UP TESTS AFTER TREATMENT

The policy of physicians for female gonorrhoea contacts in whom the initial investigations were negative was described in part I of this paper. It was indicated that in 36% of clinics investigations were repeated three or more times in these patients. It was suggested that they need not be examined more than twice after the original examination (three times altogether). The same applies to follow-up tests of cure, two sets of microscopical examinations and cultures being adequate. In all clinics at least one set of tests was carried out but in 78% of clinics seeing women three or more post-treatment tests were performed.

If more tests than necessary are being carried out new patients may be turned away who might otherwise be seen. It is suggested that instead of carrying out more than three sets of diagnostic tests in female contacts and two tests of cure, physicians should consider devoting their time to seeing new patients and taking urethral tests from asymptomatic male gonorrhoea contacts.

Conclusions

Current practices in England and Wales for the treatment and reporting of gonorrhoea have been described. It is inevitable and acceptable that there are differences in clinical management. However, in certain instances the workload is being increased unnecessarily by tests being repeated both for establishing diagnosis and cure.

The quarterly returns form the basis of statistics on gonorrhoea and are the only routine indicators

of the ability of the service to control this disease. Although it is neither desirable nor necessary to have uniform treatment schedules, it is essential that there be a uniform approach to the completion of the quarterly returns. Cases that are treated epidemiologically and are not confirmed should not be included as cases of gonorrhoea. Unless a standard approach is established consultants may continue to use different criteria and the statistics become of limited and dubious value.

This study was supported as part of a project grant from the Medical Research Council. I should like to thank Miss E. Belsey for her statistical help and Dr B. H. O'Connor for assisting me in carrying out a substantial number of interviews. I am most grateful to Dr R. D. Catterall and Professor D. L. Miller for their encouragement and support, and to Dr C. S. Nicol and Dr R. S. Morton for their comments. I am particularly grateful to Dr R. R. Willcox for his generous advice on the design of the study.

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Sexually transmitted diseases

The present situation in Sweden

JOHAN WALLIN

From the Department of Dermatology and Venereology, University Hospital, Uppsala, Sweden

Introduction

In Sweden there are strict laws stipulating that venereal diseases must be reported. However, most patients who visit clinics for sexually transmitted diseases (STDs) are not suffering from the traditional venereal diseases but from other types of STDs. These other diseases do not need to be reported and no figures are available for the country as a whole, but the STD clinic of the University Hospital in Uppsala has continuously recorded all diagnoses, as well as other factors, since 1972. There are reasons to believe that the figures for Uppsala are similar to those at other STD units in Sweden.

Gonorrhoea and syphilis in Sweden

Figure 1 shows the number of cases of gonorrhoea and syphilis in Sweden between 1950 and 1976. The

Address for reprints: J. Wallin, Department of Dermatology and Venereology, University Hospital, S-750 14 Uppsala 14, Sweden
Received for publication 1 September 1977

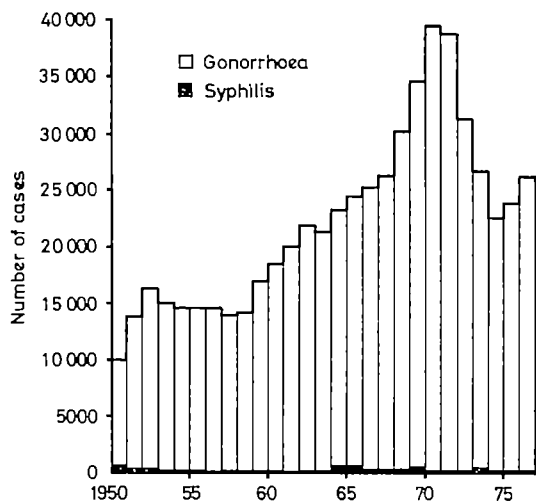


Fig. 1 The number of cases of gonorrhoea and syphilis in Sweden, 1950-76

rate for syphilis is low. The rate for gonorrhoea increased from 1959 to 1970 and then there was a marked decrease, which in the last two years has changed to a new period of increase. Table 1 shows

Table 1 Gonorrhoea in Sweden, 1975. (Cases per 100 000 inhabitants)

Age group (years)	Men	Women
10-14	2	29
15-19	482	1471
20-24	1606	1424
25-29	1040	605
Average (all ages)	312	280

the number of cases per 100 000 inhabitants according to different age groups in 1975. Figures 2 and 3 demonstrate the decrease of gonococcal infections among teenagers between 1971 and 1974. The reason for these changes is unknown but several factors have been given to explain the marked decrease during the early 1970s. More information about venereal diseases has been given at schools

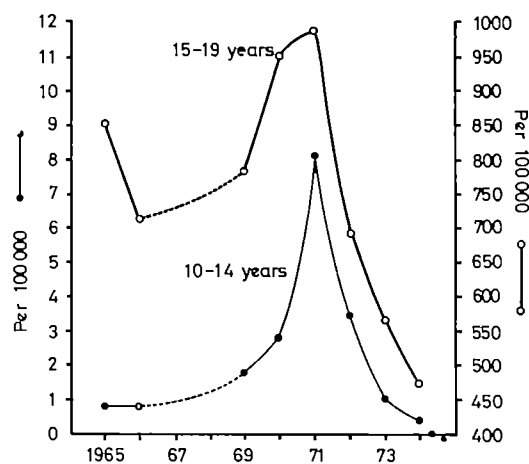


Fig. 2 The number of boys with gonorrhoea in Sweden, 1965-74.

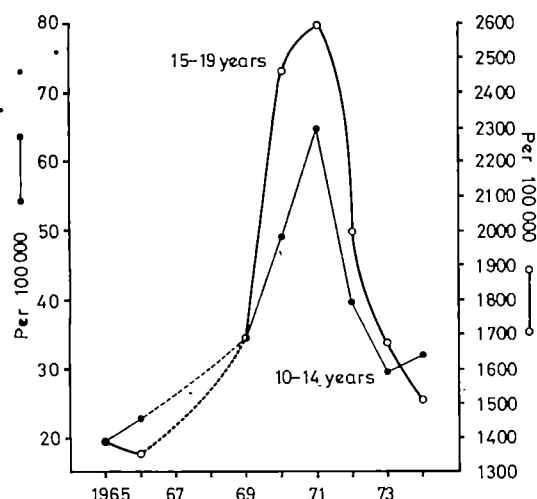


Fig. 3 The number of girls with gonorrhoea in Sweden, 1965-74.

and in newspapers. Condoms have been widely advertised. The medical facilities for managing people who suspect they have a venereal disease have improved. The treatment for gonorrhoea has been simplified, as well as the routines for examination after treatment. The relevance of each one of these factors is unknown but altogether they can be seen as part of a more open attitude towards STDs in Sweden. Infected men seem to feel more able to advise sexual partners to be examined and women do not hesitate to go to the doctor for an examination for STD. This is shown in Fig. 4 which shows a continuous decrease in the ratio of men to women per 100 000 inhabitants—from 1.7:1 in 1961 to 1:1 in 1976.

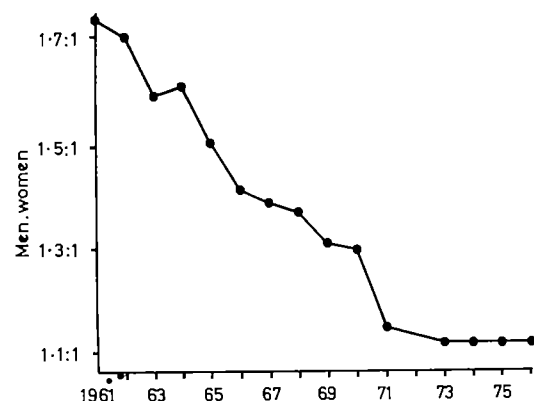


Fig. 4 Gonorrhoea in men and women in Sweden, 1961-75

When comparing two groups of patients with gonorrhoea in Uppsala in 1969 and 1974 a significant decrease was found in the percentage of men with more than one partner during the month before the day of diagnosis (Table 2). A similar

Table 2 Percentage of patients with gonorrhoea and with more than one partner during the month before the day of diagnosis

Year of investigation	Men	Women
1969	60 (n=200)	35 (n=200)
1974	42* (n=200)	28 (n=200)

* $P < 0.001$

P=The probability that the difference between the men studied in 1969 and the men studied in 1974 is caused by random factors.

comparison between figures for 1974 and 1976 shows no further decrease. This change in sexual habits in the early 1970s may help to explain the decline in gonorrhoea during that period.

Results from the STD clinic in Uppsala

Table 3 presents the final diagnoses of patients

Table 3 Final diagnoses recorded for 2181 men and 1457 women, 1972-76

Diagnosis	Men	Women
	%	%
Gonorrhoea	16.6	27.2
Syphilis	0.4	0.4
Non-gonococcal urethritis	35.1	—
Cysto-urethritis	—	3.0
<i>T. vaginalis</i> vaginitis	—	5.6
Non-specific vaginitis (including <i>C. albicans</i>)	—	26.7
Condylomata acuminata	9.3	5.8
Herpes genitalis	3.8	2.7
Pediculosis/scabies	1.7	2.4
Eczema/mycosis	2.7	0.6
Others	7.9	3.0
Free from disease	29.2	28.1

who visited the STD clinic in Uppsala from February to April in each of the years 1972-76. This clinic serves an area that has about 150 000 inhabitants including 20 000 university students. All women with a gonococcal infection diagnosed by culture at the Department of Gynaecology were followed-up in the STD clinic and are included. Gonorrhoea was diagnosed in 16.6% of the men and in 27.2% of the women. The diagnosis most often made for men was non-gonococcal urethritis (NGU), (35.1%), while in the women non-gonococcal vaginitis was found in 26.7%. Vaginitis caused by *Trichomonas vaginalis* was

found in 5.6% of the women and the relationship between vaginitis caused by *T. vaginalis* and *Candida albicans* can be estimated to be about 1:2.

Table 4 shows the types of complicated gonococcal

Table 4 *Types of gonococcal infection recorded, 1972-76*

	Men		Women	
	No.	%	No.	%
Uncomplicated	355	98.3	340	85.5
Salpingitis			53	13.4
Epididymitis	4	1.1		
Arthritis	2	0.6	0	0
Disseminated	0	0	4	1.1
Total	361	100	397	100

infection recorded during the five-year period. Salpingitis occurred in 13.4% of all women with gonorrhoea and the disseminated form was found in four, which is 1.1% of all cases of gonorrhoea in women.

The existence of *Neisseria gonorrhoeae* in the tonsillo-pharyngeal region has been a matter for conjecture for many years. In 1974, tonsillar specimens for the culturing of *N. gonorrhoeae* were taken from all patients visiting the clinic in Uppsala. In 7% of all men and 9% of all women with genital gonorrhoea, gonococci were found in the tonsillar region. Oro-genital contact was admitted by 66% of the patients with tonsillar gonococci. Despite careful questioning, no evidence was found for gonococcal transfer by mouth-to-mouth contact. Tonsillar gonorrhoea seems therefore to be of epidemiological importance mainly for those practising orogenital contact. Specimens for gonococcal culture are now taken only from selected cases, such as those in whom pharyngeal gonorrhoea or disseminated gonorrhoea is suspected. This regimen has been used for two years and so far there have been no complications from undetected pharyngeal growth of gonorrhoea.

In England an increasing number of reported cases of NGU has been found since the middle of the 1960s. There are different opinions about the reason for this increase. Is it a real increase or the result of, for example, more patients seeking medical advice and better diagnostic facilities? In Sweden, NGU is not reported and there are reasons to believe that many cases of NGU are diagnosed on inadequate grounds. As can be seen from Fig. 5, the percentage of men with NGU at the clinic in Uppsala has remained much the same for the years 1972-76. As there have been no changes in the availability of medical facilities, the diagnostic criteria used, or in the population of men during the

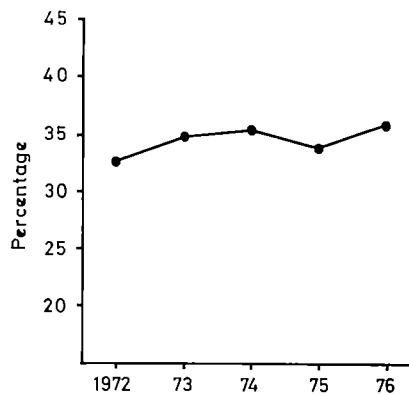


Fig. 5 *Percentage of men with NGU in Sweden, 1972-76.*

period, there is no evidence of a widespread increase in NGU in Uppsala.

Extensive studies on the importance of the *Chlamydia* organism for the development of genital infection have been undertaken in many parts of the world. Studies in Sweden do support the impression that *Chlamydia trachomatis* plays an important role as an aetiological agent for NGU (Ripa *et al.*, to be published).

Studies on acute salpingitis

A thesis on the diagnosis, aetiology, and prognosis of acute salpingitis was recently presented in Sweden (Weström, 1976). It was concluded that the causative agents in the tubal infections in acute salpingitis were still in many cases unknown, and that the rôle played by *N. gonorrhoeae* seemed to be less important now than in earlier days. Other micro-organisms, such as mycoplasmas and *Chlamydia*, could be isolated from the Fallopian tubes in women with acute salpingitis. Weström (1976) found that cultures, serological investigations, and experimental infections of organ cultures of human Fallopian tube epithelium suggest that *Mycoplasma hominis* is responsible for some cases of salpingitis. Mårdh *et al.* (1977) were able to isolate *C. trachomatis* from the cervix of 19 out of 53 women with acute salpingitis and from the Fallopian tubes of six out of 20 of these women.

Sweden and the law

In 1976 the Swedish State Social Health Board presented an analysis of the situation concerning the venereal diseases and the law. In the report it was stated that:

1. Most of the patients seeking medical advice at the special clinics for venereal disease do not have a venereal disease but another STD, or are free from disease.
2. Since 1970 there has been a marked decrease in the number of reported cases of gonorrhoea.
3. Complications after other STDs seem to be as serious as those after gonorrhoea.
4. There were few cases of infected infants reported during recent years.
5. Contact tracing by the public health officer affects only a minority of persons with gonorrhoea.
6. Treatment for gonorrhoea is simple and effective.
7. The special regulations by law point out to the public the difference between diseases in

general and venereal diseases. This may contribute to the preservation of old attitudes.

It was concluded in the report that the treatment of gonorrhoea by no means needed to be regulated by a special law. There are good reasons to believe that within a few years Sweden will reduce the number of regulations concerning venereal disease.

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Epidemiology and treatment of gonorrhoea caused by penicillinase-producing strains in Liverpool

O. P. ARYA, ELISABETH REES, A. PERCIVAL, C. D. ALERGANT, E. H. ANNELS, AND G. C. TURNER

From the Department of Venereology, Liverpool Area Health Authority (Teaching), and Mersey Regional Health Authority, the Department of Medical Microbiology, Liverpool Royal Infirmary and University of Liverpool, and the Public Health Laboratory, Liverpool

SUMMARY The epidemiological features are described of an outbreak of gonorrhoea caused by penicillinase-producing strains of gonococci in 76 patients in Liverpool between February and November 1976. Initially infections were confined to a socially deprived inner city area with a large immigrant population, and subsequent spread of infection remained circumscribed. The characteristics of those patients acquiring these infections were similar to those infected by gonococci of diminished sensitivity to benzylpenicillin, and showed a strong association with adverse social factors. A comparison of the clinical features of patients harbouring sensitive, less sensitive, and penicillinase-producing strains showed severe symptoms and signs in men and a greater involvement of multiple sites in women infected with penicillinase-producing gonococci. Treatment with penicillins failed. Tetracycline was satisfactory in men but was less so in women in whom gonococci persisted in the rectum. Cefuroxime and spectinomycin were effective.

Introduction

Penicillinase-producing gonococci appeared in Liverpool in February 1976 (Percival *et al.*, 1976; Turner *et al.*, 1976) and, simultaneously, in the Far East (Ashford *et al.*, 1976). Subsequently, they were recognised in a patient infected in West Africa (Piot, 1977). Liverpool strains have spread to other parts of the United Kingdom (Wilkinson *et al.*, 1976), Grays, Essex (Wisdom, 1977, personal communication), and Chichester (Warren-Browne, 1976, personal communication). As well as becoming established in North America (Center for Disease Control, 1977), strains from the Far East have spread to many other countries including Australia, Canada, Japan, Netherlands (Blog *et al.*, 1977), New Zealand, Norway, Philippines, Republic of Korea, and Singapore (Center for Disease Control, 1977). Earlier, the World Health Organisation warned doctors and laboratories of this development and issued recommendations for the surveillance of β -lactamase-producing gonococci (World Health Organisation, 1976).

The discovery of these strains carries grave implications (*British Medical Journal*, 1976; *Lancet*, 1976). Poor countries, especially, may lack the basic facilities required for their recognition and find their treatment expensive.

Some of our initial observations have been reported (Percival *et al.*, 1976). Here, we describe the epidemiological and clinical features of the outbreak in Liverpool, its development and control, and the response to treatment of patients infected by β -lactamase-producing gonococci.

Materials and methods

Every venereologist and bacteriologist in the sexually transmitted disease service in Liverpool and Birkenhead participated in the study. The clinics were those at the Liverpool Royal Infirmary, the Seamen's Dispensary, and St James' Hospital, Birkenhead. The clinical and epidemiological details of patients infected by β -lactamase-producing gonococci seen between February 1976 and 15 November 1976 are presented, and compared with prospectively collected data for patients with non-penicillinase-producing gonococci seen between 15 August and 15 November 1976. For comparison,

Address for reprints: O. P. Arya, Liverpool Royal Infirmary, Pembroke Place, Liverpool L3 5PU

Received for publication 7 July 1977

the non-penicillinase-producing strains are divided into two categories: those with minimum inhibitory concentrations (MICs) of benzyl penicillin of less than 0.125 units per ml, designated as 'sensitive', and those with MICs of 0.125 units per ml or above, designated as 'less sensitive'. All information was recorded on proformas. The significance of differences was determined by the χ^2 test.

The laboratory methods have been described elsewhere (Percival *et al.*, 1976; Turner *et al.*, 1976). The usual treatment for uncomplicated gonorrhoea, as soon as the provisional diagnosis based on the results from stains of genital secretions was made but before the results of culture were available, was procaine penicillin, 1.2 megaunits intramuscularly (i.m.), supplemented orally by either ampicillin 1 g, talampicillin 1 g, or amoxycillin 0.5 g or 1 g. For those who were allergic to penicillin and those in whom initial treatment failed, one of the following regimens was given: erythromycin or tetracycline 500 mg orally six hourly for 5 to 7 days, spectinomycin 2 g i.m. for men and 2 g or 4 g i.m. for women, kanamycin 2 g i.m., or co-trimoxazole four tablets twice daily for two days. From the middle of October, cefuroxime in a single dose of 1 g i.m. was used. Cure of infection was judged by negative culture at least twice, and usually three times, during three weeks after the end of treatment. In women this included rectal as well as urethral and cervical cultures.

Results

At two of the three clinics (Liverpool Royal Infirmary and the Seamen's Dispensary), 408 patients were studied. There were 267 men, including 44 with penicillinase-producing isolates, and 141 women, including 32 with enzyme positive isolates. The findings at the third clinic (St James' Hospital, Birkenhead), serving the Wirral, are described separately.

EPIDEMIOLOGY

Age and marital state

There were no significant differences in age or marital state between the three groups.

In Tables 1, 2, and 3, a number of epidemiological characteristics are presented for men and women separately, according to the sensitivity to benzylpenicillin of the gonococci isolated. The contingency tables with their χ^2 values are shown separately. Those for whom the relevant information was not available are excluded from the calculations of percentages and χ^2 values. The contingency tables relating to Table 1 are designated as Tables 1a, 1b, and 1c, the table relating to Table 2 is

designated as Table 2a, and those relating to Table 3 are labelled consecutively from Table 3a to Table 3h.

Race (Tables 1 and 1a)

Of the β -lactamase positive isolates 76% were from Black and only 18% were from White men. This ratio was almost completely reversed among 'sensitive' isolates with 17% from Blacks and 76% from Whites. This difference was highly significant ($P < 0.001$). In contrast, only a few women in all three groups were Black.

History of gonorrhoea (Tables 1 and 1b) and no. of consorts (Tables 1 and 1c)

In men there were significant differences between those with penicillinase-producing and 'sensitive'

Table 1 Epidemiological characteristics of patients related to sensitivity to benzylpenicillin of infecting gonococci

Characteristics	No. positive related to sensitivity of isolate*					
	β -Lactamase negative				β -Lactamase positive	
	Sensitive		Less sensitive			
	Male	Female	Male	Female	Male	Female
	No. %	No. %	No. %	No. %	No. %	No. %
Race						
White	117 76	68 91	33 48	28 90	6 18	14 93
Black	27 17	4 5	33 48	3 10	26 76	1 7
Coloured	11 7	3 4	2 3	—	2 6	—
Not known	—	—	—	—	10 —	17 —
Previous gonorrhoea	42 28	13 17	23 38	5 16	22 50	7 22
Consorts						
2 or more	46 30	14 18	21 32	5 16	19 54	10 33
No. studied in each category	155	78	68	31	44	32

*Sensitive = MIC of benzylpenicillin below 0.125 units/ml

Less sensitive = MIC 0.125 units/ml or above

Table 2 Occupation of patients related to sensitivity to benzylpenicillin of infecting gonococci

Occupation	No. positive related to sensitivity of isolate*					
	β -Lactamase negative				β -Lactamase positive	
	Sensitive		Less sensitive			
	Male	Female	Male	Female	Male	Female
	No. %	No. %	No. %	No. %	No. %	No. %
Skilled or professional	53 34	20 26	15 22	7 23	9 20	6 19
Unskilled or unemployed	50 32	33 42	22 33	13 42	26 59	12 37
Student	12 8	5 6	6 9	1 3	5 11	2 6
Seaman	19 12	—	18 27	—	1 2	—
At home	—	20 26	—	10 32	—	12 38
Others	21 14	—	7 10	—	3 7	—
No. in each category	155	78	68	31	44	32

*Sensitive = MIC of benzylpenicillin below 0.125 units/ml

Less sensitive = MIC 0.125 units/ml or above

Table 3 *Residence of patients and consorts related to sensitivity to benzylpenicillin of infecting gonococci*

Residence	No. positive related to sensitivity of isolate*					
	β -Lactamase negative				β -Lactamase positive	
	Sensitive		Less sensitive			
	Male	Female	Male	Female	Male	Female
No. %	No. %	No. %	No. %	No. %	No. %	No. %
Of patients						
Liverpool						
7 and 8	29 19	12 15	29 43	16 52	25 57	16 50
Other						
Liverpool	93 60	56 72	21 31	14 45	16 36	16 50
Wirral	7 4	7 9	2 3	—	1 2	—
UK	14 9	3 4	3 4	1 3	1 2	—
Foreign	12 8	—	13 19	—	1 2	—
Of consorts or meeting place						
Liverpool						
7 or 8	11 7	12 16	29 43	15 50	11 33	16 55
Other						
Liverpool	90 58	48 66	26 38	13 42	17 52	10 35
Wirral	4 3	5 7	—	—	—	1 3
UK	34 22	8 11	4 6	2 7	4 12	1 3
Foreign	16 10	—	9 13	—	1 3	1 3
Liverpool unspecified	—	5 —	0 —	1 —	11 —	3 —
No. in each category	155	78	68	31	44	32

*Sensitive = MIC of benzylpenicillin below 0.125 units/ml
 Less sensitive = MIC 0.125 units/ml or above

Contingency tables 1a-3d *Comparisons between patients infected by β -lactamase producing and sensitive gonococci*

Epidemiological characteristics	No. positive related to sensitivity of isolate*		χ^2 with Yates's correction	P
	Sensitive	β -Lactamase+		
Race (men) (Table 1a)				
White	117	6	45.67	<0.001
Black	27	26		
Previous gonorrhoea (men) (Table 1b)				
Yes	42	22	6.34	<0.02
No	107	22		
No. of consorts (men) (Table 1c)				
2 or more	46	19	6.08	<0.02
Less than 2	105	16		
Occupation (men) (Table 2a)				
Unskilled or unemployed	50	26	9.35	<0.01
Others	105	18		
Patient's residence (Table 3a)				
Men (Table 3a)				
Liverpool 7 and 8	29	25	23.28	<0.001
Others	126	19		
Women (Table 3b)				
Liverpool 7 and 8	12	16	12.56	<0.001
Others	66	16		
Consort's residence or meeting place (Table 3c)				
Men (Table 3c)				
Liverpool 7 and 8	11	11	15.68	<0.001
Others	144	22		
Women (Table 3d)				
Liverpool 7 and 8	12	16	13.75	<0.001
Others	61	13		

*Sensitive = MIC of benzylpenicillin below 0.125 units/ml

isolates with respect to history of gonorrhoea (50% compared with 28%, $P < 0.02$) and having two or more consorts during the three weeks before attending the clinic (54% compared with 30%, $P < 0.02$).

Occupation (Tables 2 and 2a)

Many of the men infected by enzyme positive gonococci were unskilled or unemployed (59%) compared with those infected by 'sensitive' gonococci (32%, $P < 0.01$). Only one of the men with enzyme positive isolates was a seaman. Five were students, all being foreigners from Africa or the Middle East but living in Liverpool.

Residence of patients (Tables 3, 3a, 3b, 3e, and 3f)

A high proportion of both enzyme positive and 'less sensitive' isolates occurred in men and women who lived in only two (7 and 8) of the 36 districts of Liverpool and adjacent areas. Thus, approximately half of all isolates in these two groups were found in such patients, but only 15% and 19% respectively for women and men with 'sensitive' isolates ($P < 0.001$). Only two men with penicillinase-producing isolates came from outside the Liverpool area; one, a West Indian from Birmingham, had acquired his infection in Liverpool and the other, a seaman from Ghana, claimed to have last had sexual intercourse in Germany. He arrived in Liverpool at least a week after the discovery of the other patients with enzyme positive gonococci.

Contingency tables 3e-3h *Comparisons between patients infected by sensitive and less sensitive gonococci*

Epidemiological characteristics	No. positive related to sensitivity of isolate*		χ^2 with Yates's correction	P
	Sensitive	Less sensitive		
Patient's residence				
Men (Table 3e)				
Liverpool 7 and 8	29	29	12.86	<0.001
Others	126	39		
Women (Table 3f)				
Liverpool 7 and 8	12	16	13.41	<0.001
Others	66	15		
Consort's residence or meeting place				
Men (Table 3g)				
Liverpool 7 and 8	11	29	38.20	<0.001
Others	144	39		
Women (Table 3h)				
Liverpool 7 and 8	12	15	10.71	<0.01
Others	61	15		

*Sensitive = MIC of benzylpenicillin below 0.125 units/ml
 Less sensitive = MIC of benzylpenicillin 0.125 units/ml or above

Residence of consorts or meeting place (Tables 3, 3c, 3d, 3g, and 3h)

The same differences were observed in consorts' address or meeting place. For men, 33% of consorts

of those infected by enzyme positive isolates and 43% of those with 'less sensitive' isolates, but only 7% of those with 'sensitive' isolates either lived or were met in Liverpool 7 or 8 (respectively, $P < 0.001$ and $P < 0.001$). For women, the corresponding figures were, 55%, 50%, and 16% (respectively $P < 0.001$ and $P < 0.01$). Two women infected by penicillinase-producing gonococci in March 1976, had additional consorts in London, York, and Chichester. These consorts had failed treatment with penicillin without recognition of penicillinase production. Four men infected in Liverpool had additional consorts elsewhere, three in London and one in Middlesbrough.

One club, used by non-Whites, was often named as the meeting place for those with 'less sensitive' and penicillinase-producing gonococci.

Sixteen per cent of the women infected by enzyme positive gonococci, all unmarried, but only 4% of those with 'sensitive' gonococci were pregnant when first seen, suggesting a less responsible approach to contraception in the former category.

CLINICAL FEATURES

The clinical features on first presentation for men are shown in Table 4 and for women in Table 5. In men the only significant difference was the higher incidence (23%) of enlarged tender inguinal lymph glands in those infected by enzyme positive gonococci than in either of the other two categories (2 and 4%, $P < 0.001$). In women, penicillinase-producing gonococci were more often isolated from each site and from multiple sites and associated with

Table 5 Clinical features in women related to sensitivity to benzylpenicillin of infecting isolate

Clinical features	No. positive related to sensitivity of isolate*					
	β -Lactamase negative				β -Lactamase positive	
	Sensitive		Less sensitive			
	No.	%	No.	%	No.	%
Symptoms						
None	40	51	9	29	18	56
Discharge	15	19	8	26	6	19
Abdominal pain	10	13	7	23	5	16
Others	13	17	7	23	3	9
Duration						
<1 week	10	26	5	23	7	50
1 to 2 weeks	15	39	10	45	2	14
>3 weeks	13	34	7	32	5	36
Signs						
None	25	32	10	32	11	34
Cervicitis	16	20	3	10	3	9
Ubr†	6	8	3	10	4	12
Multiple	16	20	9	29	7	22
Salpingitis	8	10	4	13	5	16
Others	7	9	2	6	2	6
Positive culture						
Cervix	68	87	25	81	30	94
Urethra	50	64	23	74	27	84
Rectum	26/57	46	16/25	64	12/20	60
For trichomoniasis	9	12	6	19	8	25
No. in each category	78		31		32	

*Sensitive = MIC of benzylpenicillin below 0.125 units/ml

Less sensitive = MIC 0.125 units/ml or above

†Ubr = mucopus in the urethra or Bartholin duct or rectum

trichomonal infection than in the other two groups, but the differences were not statistically significant.

Only one patient, a man infected by a penicillinase-producing gonococcus, developed disseminated infection characterised by rash and arthritis, three days after treatment with penicillin.

RESPONSE TO TREATMENT

In assessing response to treatment, the usual difficulty of distinguishing relapse from reinfection was even greater for those with penicillinase-producing isolates because of what appeared to be the higher level of promiscuity. Table 6 shows the response to the various treatments in those adequately followed-up. Those considered reinfected and a patient with a penicillinase-positive isolate who received further medication from a doctor for bronchitis are excluded.

Men

There were only three (2.7%) of 110 with sensitive strains and six (14.3%) of 42 with less sensitive isolates who failed treatment with penicillins. One of the latter might have had a penicillinase-positive infection unrecognised in the laboratory, because this was observed in his consort. In the penicillinase-positive group, 36 (95%) of 38 treatments failed. One of the remaining two patients was treated for post-gonococcal urethritis, without further culture

Table 4 Clinical features in men related to sensitivity to benzylpenicillin of infecting gonococci

Clinical features	No. positive related to sensitivity of isolate*			
	β -Lactamase negative		β -Lactamase positive	
	Sensitive		Less sensitive	
	No.	%	No.	%
Symptoms				
Discharge	84	54	36	53
Discharge + dysuria	57	37	27	40
Others	9	6	3	4
None	5	3	2	3
Duration				
<1 week	121	81	56	85
1 to 2 weeks	26	17	10	15
>3 weeks	3	2	—	—
Signs				
Purulent discharge	136	88	61	90
Discharge + inguinal lymphadenopathy	3	2†	3	4
No. in each category	155		68	

*Sensitive = MIC of benzylpenicillin below 0.125 units/ml

Less sensitive = MIC 0.125 units/ml or above

† $P < 0.001$

Table 6 *Results of treatment related to sensitivity to benzylpenicillin of infecting gonococci*

Treatment†	β -Lactamase negative*				β -Lactamase positive	
	Sensitive		Less sensitive			
	Followed	Failed	Followed	Failed	Followed	Failed
Penicillins						
Men	110	3 (2.7%)	42	6 (14%)	38	36 (95%)
Women	52	1 (1.9%)	18	0	18	17 (94%)
Tetracycline						
Men	9	0	1	0	9	0
Women	16	2 (12.5%)	5	1	10	3 (30%)
Spectinomycin						
Men	4	1	6	0	18	1 (5.5%)
Women	1	0	0		5	0
Erythromycin						
Men	0		1	0	2	2
Women	1	0	0		6	1
Kanamycin						
Men	0		1	0	0	
Women	2	0	0		1	0
Cefuroxime						
Women	0		0		8	0
Co-trimoxazole						
Men	2	1	0		2	0

*Sensitive = MIC of benzylpenicillin below 0.125 units/ml

Less sensitive = MIC of benzylpenicillin 0.125 units/ml or above

†See text for details

because the smear was negative. The other remained apparently cured for up to two weeks after treatment and then he defaulted.

The number of patients treated with the other regimens was small. For all three categories of gonococci, only two of 28 patients failed treatment with spectinomycin and none of 19 with tetracycline. Both patients with penicillinase-positive isolates who received erythromycin failed treatment.

Women

For patients with enzyme negative isolates, penicillins gave better results in women than men. One patient with an apparent cure among the penicillinase-positive patients was given a course of tetracycline at her second visit, before her negative culture result became known. With tetracycline, five of the six failures, including three with penicillinase-positive isolates, were persistently positive only in the rectum. It is possible there were two more failures in the penicillinase-positive group. Repeated follow-up tests showed the strains to be enzyme negative suggesting reinfection, but the possibility of loss of the penicillinase determining plasmids by the infecting organism could not be excluded. Eight patients harbouring penicillinase-positive strains were cured with cefuroxime. These included a woman with salpingitis who was treated successfully with cefuroxime 0.5 g every eight hours for five days.

FINDINGS AT BIRKENHEAD

Only three of 61 patients studied at St James' Hospital, Birkenhead, were proved to be harbouring penicillinase-producing gonococci. The first, a woman, was seen on 25 October 1976. All three failed treatment with penicillins, but one responded to spectinomycin and the other two to cefuroxime.

Discussion

EPIDEMIOLOGY

In this study, penicillinase-producing gonococci in men were generally in those aged between 20 and 29 years old, who were Black and single. In addition either the patient or his consort lived in or frequented clubs in Liverpool 7 or 8. He was unskilled, unemployed, promiscuous, and had had gonorrhoea once or more in the past. Women with enzyme positive isolates were usually aged between 15 and 29 years, and were White, single or separated. The patient or her consort lived in or frequented clubs in Liverpool 7 or 8, did not regularly practise contraception, was unemployed, promiscuous (although not the same extent as the man), and cohabited with known consorts. She was likely to default and was evasive.

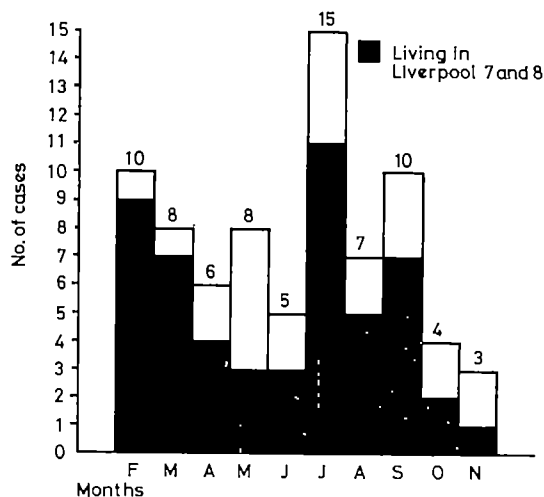


Figure Monthly distribution of cases

The figure shows the total monthly distribution of patients with penicillinase-positive isolates and the proportion of those who lived or acquired their infection in Liverpool 7 or 8. Penicillinase-positive isolates appeared in early February 1976. There were 10 infected patients that month and nine of them lived in Liverpool 7 or 8. Their consorts also either lived in or were met in clubs in the same area (apart from an additional consort in London).

They either presented later or were not seen at all. The remaining patient was the Ghanaian sailor who denied having sexual intercourse in Liverpool and arrived at least one week after the first patients with penicillinase-producing gonococci were seen. Many of the same characteristics were common to those harbouring the less sensitive isolates. These observations show that, in Liverpool, the occurrence of most gonococcal infections is relatively circumscribed, affecting members from the same areas with similar social backgrounds and recreational meeting places. Association of coloured immigrants with a high incidence of gonorrhoea in the United Kingdom (Willcox, 1966; Oller and Wood, 1970) and association with high penicillin MICs (Silver and Darling, 1971) have been noted.

Remarkably, it was eight months before an enzyme positive isolate was found in Birkenhead, separated from Liverpool by only the Mersey River. However, patients attending this clinic differed from those seen in Liverpool. Only one coloured man with a local address had gonorrhoea during the study period. His strain was reported to be sensitive to penicillin but this drug failed to cure his infection. His recent consort proved to be harbouring a penicillinase-positive gonococcus and had earlier had sexual intercourse at a party in Liverpool 8. The female partner of the other two patients in Birkenhead was known to have had gonorrhoea at least twice before and she had associations with Liverpool 8. While 30% of the isolates in Liverpool were 'less sensitive' to benzylpenicillin, only 12% of those at Birkenhead were in this category.

Large towns generally have areas where venereal disease is prevalent. Liverpool is clearly no exception, with some clubs in the inner city being places of high risk. Liverpool 8, especially, contains large areas that have the worst social conditions in the city (Department of the Environment, 1977; Liverpool City Planning Officer 1977, personal communication). The characteristics are those of multiple deprivation—including high rates of sickness and infectious diseases, overcrowding, large families, many immigrants, a large number of people aged between 15 and 24, long-term unemployment, job instability, high rates of infant mortality, illegitimacy and delinquency, and the issuing of supervision orders.

Less frequently the penicillinase-producing gonococci escaped from Liverpool. Two women who defaulted had allegedly been commuting between Glasgow and London via Liverpool. As a result at least one man, a Black seaman who was seen at the Seamen's Dispensary at the end of November 1976, seemed to have acquired a

penicillinase-producing gonococcus in Glasgow. Another woman, presenting in February 1977, claimed to have acquired the infection in London from a seaman recently returned from the Far East and she denied further sexual intercourse for three weeks, but spermatozoa were observed in the vaginal smear. Such an instance illustrates the well-known unreliability of some patients' information and the impossibility of determining the origin of the penicillinase-producing gonococci in Liverpool without epidemiological markers for the isolated gonococci.

ORIGIN OF THE PENICILLINASE-PRODUCING GONOCOCCI ISOLATED IN LIVERPOOL

Except for the Ghanaian sailor (the only seaman), all patients seemed to have acquired the penicillinase-producing gonococci in Liverpool. None admitted to recent sexual intercourse outside Liverpool. Another early case with a high MIC (more than 0.5 units per ml, enzyme not tested) in whom treatment failed with penicillin was that of a Nigerian. He had not left Liverpool since September 1975 and had had sexual intercourse in Liverpool, 12 days before attending the clinic; he denied any other sexual contact in the preceding weeks. Thus, the penicillinase-producing gonococci isolated in Liverpool could have arisen in Liverpool itself. As yet, the only other known possible source is Ghana. Nine gonococci isolated in America, and apparently acquired in the Far East, have been shown to possess plasmids of a molecular weight that was different from that of the three Liverpool isolates (Roberts and Falkow, 1977). However, the one isolated in London from a Ghanaian woman (Phillips, 1976), had a plasmid similar to that found in the Liverpool isolates. The London isolate and one from a Swedish sailor returning from Ghana were of the same auxotype as all the Liverpool isolates tested by one of us (AP). Therefore, it appears that all the Liverpool isolates were the same organisms and may prove to be identical with the two from Ghana, but there is no information about the time of emergence or prevalence of enzyme producing gonococci in Ghana to suggest their origin. Apparently none of the 80 isolates recently tested in Ibadan, Nigeria, was an enzyme producer (Osoba, 1977, personal communication).

CLINICAL FEATURES AND TREATMENT

In men, the few differences between those harbouring the penicillinase-producing gonococci and the rest—a shorter incubation period, slightly more severe symptoms, and a higher incidence of regional adenitis—suggest a greater tendency to local invasiveness. Only one patient with an enzyme positive strain developed disseminated infection.

We have not yet encountered a homosexual with rectal gonorrhoea caused by a penicillinase-producing gonococcus. One of the likely reasons is that most Black patients do not have rectal intercourse.

In women the symptoms and signs, including those suggesting salpingitis, were similar among the penicillinase-positive and penicillinase-negative categories. However, gonococci were isolated more often from multiple sites and this might indicate enhanced local invasiveness of penicillinase-positive gonococci.

While the treatment results with penicillin were as expected, the results of treatment with tetracycline in women were poor. The isolates were not fully sensitive to tetracycline (MICs 1 or 2 µg/ml). Serum levels in a group of women in Liverpool being studied by one of us (ER), receiving tetracycline 500 mg every six hours for chlamydial infection, rarely exceeded or even reached 2 µg/ml. For such relatively insensitive gonococci our results suggest that the failure rate with tetracycline is unacceptably high for women but not for men. Persistently positive post-treatment cultures from the rectum emphasise the necessity of taking tests from all potentially infected sites, otherwise one may miss not only the penicillinase-producing strains (Percival *et al.*, 1976) but also treatment failures in women.

The number of patients treated with the other regimens was small. However, spectinomycin and cefuroxime appeared to be effective in both men and women.

Control of the outbreak

Apart from one man seen at the end of November 1976 (included in Table 7), no new patient harbouring penicillinase-producing gonococci was seen until February 1977 when infection was discovered in a woman. There were two more cases in women in April. Consorts of two of these women were seamen who were not seen. They either could have reintroduced penicillinase-positive gonococci into Liverpool or the organisms might have remained asymptotically in them and been acquired earlier in Liverpool. The gonococcus was isolated in the third woman because her baby developed conjunctivitis shortly after birth. She was unmarried and claimed not to have had sexual intercourse for eight months. All three isolates were of the same auxotype as those in 1976.

During 1976 several patients, mainly women, had defaulted for long periods either untreated or with unknown outcome of treatment, despite intensive efforts in contact tracing, including up to 15 visits

Table 7 Relationship between no. of new and untreated patients infected by penicillinase-producing gonococci

Year	Month	New patients in each month		Cumulative numbers remaining untreated at end of each month	
		Total	Women	Total	Women
1976	February	10	2	4	
	March	8	4	5	4
	April	6	3	4	3
	May	8	2	2	1
	June	5	3	3	3
	July	15	4	6	3
	August	7	3	4	3
	September	10	6	6	6
	October	4	3	4	4
	November	4	2	3	2
	December	0	0	0	0
1977	January	0	0	0	0
	February	1	1	1	1
	March	0	0	0	0
	April	2	2	0	0
	May	0	0	0	0

to their homes. In Table 7, the number of new patients infected by penicillinase-producing gonococci each month is related to the cumulative numbers, at the end of the same month, of patients with enzyme positive gonococci who remained in Liverpool and were known to have been inadequately treated or were still untreated. It can be seen that the outbreak was apparently and at least temporarily controlled only when known cases had been effectively treated or the patient had left the area.

During the episode, 62% of declared consorts were traced and reported for treatment. Of the remaining 38%, some were probably treated at other clinics or at our clinics without an association with known cases being disclosed.

Four other factors may have contributed to limiting the outbreak. Firstly, in October, the change in second line treatment from tetracycline to cefuroxime as well as spectinomycin resulted in cures being achieved more quickly in women. Secondly, as most of the failures in women were confined to the rectum, theoretically at least, infectivity would be low in the absence of rectal intercourse, a practice believed to be rare in women. Thirdly, the gonococci could have spontaneously lost the plasmid determining production of penicillinase. In two patients only non-penicillinase-producing strains were isolated after treatment with penicillin and in one other this happened without any treatment, but it could have been a sampling error. Fourthly, unlike some of those found in the USA, Liverpool isolates do not carry the larger plasmid necessary for transfer of the penicillinase determining plasmid to other gonococci (Roberts and Falkow, 1977) as could occur in mixed infections.

Any long-term planning for the control of these strains cannot be separated from the control of gonorrhoea in general and possibly of other sexually transmitted diseases. A special situation, however, may be said to exist in some socially deprived areas of Liverpool with a predominantly immigrant and student population, which seems to be associated with the less sensitive and resistant strains of gonorrhoea. The relationship of venereal disease to adverse social phenomena is well known (Morton, 1973). Furthermore, a penicillinase-producing strain could at any time be reintroduced into an area by a traveller from other parts of the world where such strains continue to be common. Therefore, vigilance, appropriate treatment, and determined contact tracing must continue to remain the principal lines of defence.

We are indebted to Dr A. K. Ghosh, Dr P. B. Carey, Dr Jean C. Davies, Dr I. Anne Tait, Dr S. S. Pareek, Dr Joyce Angami, and Dr A. Hooi for their clinical assistance. Equally important was the contribution of the nursing, social services, contact tracing, technical, clerical, and secretarial staff. We thank Professor Ian Phillips and Dr I. Moberg for the subcultures of penicillinase-producing gonococci. One of us (AP) is grateful to the Medical Research Council for financial support.

Addendum

Since this paper was submitted, two more cases, seen in Liverpool in January 1978, have been found to be infected with β -lactamase-producing gonococci. One, a woman with salpingitis, was infected by a Ghanaian seaman between October and December 1977 and the other, a Sudanese seaman acquired his infection in Port Sudan.

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Cultural diagnosis of gonorrhoea with modified New York City (MNYC) medium

H. YOUNG

From the Department of Bacteriology, University Medical School, Edinburgh

SUMMARY A simply prepared modified New York City medium, designated MNYC was compared with Thayer Martin (TM) medium for the cultural diagnosis of gonorrhoea. MNYC medium contained lincomycin, commercial gonococcal base and lysed whole blood, whereas the original New York City medium contained fresh horse plasma and haemoglobin solution, a basal medium prepared from basic ingredients and vancomycin. Using MNYC medium gonococci were cultured from 96.1% of men and 100% of women with gonorrhoea (positive film and/or culture) compared with only 77.6% and 69% respectively using TM medium. There were no patients positive by culture on TM medium but negative by culture on MNYC medium. The proportion of men with positive films but negative culture was reduced from 17.1% on TM medium to 3.9% on MNYC medium. There were no women with positive films but negative cultures on MNYC medium compared with 19% on TM medium. MNYC medium is recommended as a simply prepared and highly efficient medium for the cultural diagnosis of gonorrhoea.

Introduction

Although microscopical examination is important in making a presumptive diagnosis of gonorrhoea so allowing immediate treatment, cultures are obligatory in the diagnosis of rectal, oral, disseminated, and asymptomatic infections in both sexes. They are also essential in determining antibiotic sensitivities and for evaluating treatment.

The selective medium of Thayer and Martin (1966) is widely used in many laboratories and has increased the number of positive isolates from all sites, but it has proved particularly valuable in isolating the gonococcus from heavily contaminated sites such as the rectum (Roepstorff and Hammarström, 1966). Unfortunately, at least 3% of gonococcal strains are sensitive to vancomycin at a concentration of 3 µg/ml (Reyn, 1969) and are therefore unlikely to be detected when Thayer Martin (TM) medium, containing vancomycin 5 µg/ml, is used. Also, isolates tend to grow slowly on TM medium and produce small colonies.

On introducing delayed immunofluorescence for the routine identification of gonococci we found that

slow growth on TM medium became the limiting factor in the speed of cultural diagnosis. Faur *et al.* (1973a, b) described a new selective medium, designated NYC (New York City) medium, which provided luxuriant growth of pathogenic neisseriae after incubation for 24 hours. NYC medium essentially consists of a proteose peptone-corn starch agar buffered base to which is added a haemoglobin solution prepared from fresh horse erythrocytes, with horse plasma, yeast dialysate, glucose and the antibiotics—vancomycin, colistin, amphotericin B, and trimethoprim lactate.

The aim of this investigation was to compare culture results obtained using our standard TM medium with those obtained using a simply prepared modification of NYC medium.

Material and methods

MEDIA

TM medium comprised lab m Columbia agar base (London Analytical and Bacteriology Media Ltd, 50 Mark Lane, London) supplemented with 10% (by vol.) heated (56°C for 60 min) human blood and the antibiotics vancomycin (4 µg/ml), colistin (6 µg/ml), and nystatin (10 µg/ml).

MNYC (Modified New York City) medium A comprised Difco gonococcal base enriched with

Address for reprints: H. Young, Department of Bacteriology, University Medical School, Edinburgh

Received for publication 26 July 1977

10% (by vol.) defibrinated horse blood (Wellcome Ltd) lysed with 0.5% (by vol.) saponin, 2.5% (by vol.) yeast dialysate prepared as described by Faur *et al.* (1973a), 0.1% (by vol.) glucose, lincomycin (0.5 µg/ml), colistin (6 µg/ml), amphotericin B (1.0 µg/ml), and trimethoprim lactate (6.5 µg/ml).

MNYC medium B was prepared as above but with lincomycin at a concentration of 1.0 µg/ml.

MNYC medium C was prepared as medium B but contained 10% (by vol.) human blood, lysed with 0.5% (by vol.) saponin, in place of defibrinated horse blood.

In the first part of the trial 'split' plates were made by pouring 10 ml of MNYC medium A into one half and 10 ml of TM medium into the other half of diametrically partitioned and vented Petri plates.

In the second part of the trial split plates contained MNYC medium B and MNYC medium C.

Determination of minimum inhibitory concentration (MIC) to vancomycin and lincomycin

At the time of isolation, a suspension of each gonococcal isolate was made in skimmed milk (10%), quick frozen with a mixture of solid carbon dioxide and acetone and stored at -20°C. Later, strains were recovered and the MICs to vancomycin and lincomycin determined using sensitivity test agar (Oxoid Ltd) supplemented with 10% lysed human blood: vancomycin and lincomycin were added at concentrations of 2, 4, 8, 16, and 32 µg/ml.

PATIENTS

The patients in this study attended the Department of Venereology, Edinburgh Royal Infirmary, between 15 November 1976 and 21 February 1977.

In men, if a urethral discharge was present, or if there was presumptive evidence of contact with gonorrhoea, a Gram-stained smear of urethral discharge was examined microscopically and material inoculated directly on to culture plates as described below. In the case of homosexuals, material from the urethra, rectum and pharynx was cultured routinely, and, if negative on the first occasion, cultures were repeated twice at weekly intervals.

In women, Gram-stained smears of material from the urethra and cervix, and cultures from the urethra, cervix, rectum and, if indicated, from the pharynx, were taken. If the first cultures for *N. gonorrhoeae* were negative, these were generally repeated twice at weekly intervals.

In the first part of the study, 15 November until 31 December 1976, all specimens for *N. gonorrhoeae*, including tests of cure when required, were plated directly on to both TM medium and MNYC medium A: the order of inoculation of the medium alternated with each patient. During this period

there were 256 women yielding 2124 cultures and 422 men yielding 600 cultures.

In the second part of the study, 13 January until 21 February 1977, when cultures were made on MNYC medium B and MNYC medium C there were 287 women and 417 men.

INCUBATION AND IDENTIFICATION

After inoculation, plates were held at 36°C and transferred to the laboratory within a few hours where they were incubated at 36°C in a carbon dioxide enriched (10%) atmosphere. After incubation for 24 hours, plates were examined and any suspect colonies tested for the oxidase reaction and Gram smears prepared. Oxidase positive Gram-negative diplococci were identified by the rapid carbohydrate utilisation test and by delayed immunofluorescence with Difco fluorescein-labelled anti-gonococcal conjugate as described by Young *et al.* (1976): all isolates of *N. gonorrhoeae* produced acid from glucose only and gave a positive fluorescent antibody test. Negative cultures were re-examined after incubation for 48 hours.

Results

STUDIES WITH MNYC MEDIUM A

This medium contained 10% horse blood and lincomycin (0.5 µg/ml).

FEMALE PATIENTS

The numbers of specimens positive for *N. gonorrhoeae* by culture on TM medium are compared with those on MNYC medium A in Table 1.

Table 1 Results of culture for *Neisseria gonorrhoeae* when 2124 specimens from 256 female patients were plated on TM medium and MNYC medium A

Site	No. sampled	No. of cultures positive	
		On TM medium	On MNYC medium A
Urethra	683	16	29
Cervix	681	29	40
Rectum	586	5	8
Throat	160	2	2
Other	14*	4†	7
Total	2124	56	86

*Bartholin's gland (11), high vaginal swab (1), wrist pustule (1), coil (1)

†All Bartholin's gland

The 56 cultures positive on TM medium corresponded to 29 patients while the 86 cultures positive on MNYC medium A corresponded to 42 patients. Results of culture and microscopical examination for these patients are shown in Table 2.

Table 2 *Results of microscopical examination and culture on TM medium and MNYC medium A for 42 female patients with gonorrhoea*

Pattern of results	Patients with gonorrhoea and pattern of results			
	On TM medium		On MNYC medium	
	No.	(%)	No.	(%)
Culture+smear+	20	(47.6)	28	(66.7)
Culture+smear-	6	(14.3)	9	(21.4)
Culture+smear 0	3	(7.1)	5	(11.9)
Culture-smear+	8	(19.0)	0	(0.0)
Total	37	(88.0)	42	(100)

+ = Positive, - = negative, 0 = no smear

Of the 13 patients with gonorrhoea detected by culture on MNYC medium A but missed by culture on TM medium, eight were smear positive, three were smear negative, and there were no corresponding smears for two patients. Smears were positive but TM cultures negative in 19.0% (8/42) of patients with gonorrhoea. There were no positive smears with negative cultures when MNYC medium A was used. Microscopical examination detected 66.7% (28/42) of the women with gonorrhoea. Excluding the five patients from whom no smears were available, microscopical examination detected 75.7% (28/37) of infected patients. A combination of microscopy and culture on TM medium detected 88% (37/42) of the positive patients whereas culture on TM medium alone detected only 69.0% (29/42).

MALE PATIENTS

The numbers of specimens positive for *N. gonorrhoeae* by culture on TM medium are compared with those on MNYC medium A in Table 3.

Table 3 *Results of culture for Neisseria gonorrhoeae when 600 specimens from 422 male patients were plated on TM medium and MNYC medium A*

Site	No. sampled	No. of cultures positive	
		On TM medium	On MNYC medium A
Urethra	394	53	69
Rectum	43	3	4
Throat	152	0	0
Other	11*	0	0
Total	600	56	73

*Prostatic secretion (3), urine thread or deposit (7), eye swab (1)

As only one culture site was positive for each patient there were 56 patients with gonorrhoea detected by TM culture and 73 patients detected by culture on MNYC medium A. Results of culture and microscopical examination for these patients

and three patients from whom positive smears but negative cultures were obtained are shown in Table 4.

Table 4 *Results of microscopical examination and culture on TM medium and MNYC medium A for 76 male patients with gonorrhoea*

Pattern of results	Patients with gonorrhoea and pattern of results			
	On TM medium		On MNYC medium	
	No.	(%)	No.	(%)
Culture+smear+	40	(52.6)	53	(69.7)
Culture+smear-	8	(10.5)	10	(13.2)
Culture+smear 0	8	(10.5)	10	(13.2)
Culture-smear+	13	(17.1)	3	(3.9)
Total	69	(90.8)	76	(100.0)

+ = Positive, - = negative, 0 = no smear

Of the 17 patients giving positive cultures only on MNYC medium A 13 were smear positive, two were smear negative, and in two instances no smears were available. There were no patients giving positive cultures only on TM medium. Smears were positive but cultures negative in 17.1% (13/76) of patients when TM medium was used compared with only 3.9% (3/76) using MNYC medium A. Microscopical examination detected 73.7% (56/76) of all patients with gonorrhoea. Excluding the 10 patients from whom no smears were available, microscopical examination detected 84.8% (56/66) of cases. A combination of microscopy and culture on TM medium detected 90.8% (69/76) whereas culture on TM medium alone detected only 77.6% (56/76). Culture on MNYC medium alone detected 96.1% of men with gonorrhoea.

BOTH MALE AND FEMALE PATIENTS

Rapidity of growth

Colonies of Gram-negative diplococci were present after incubation for 24 hours in 51.8% (29/56) of the cultures positive in women and 42.9% (24/56) of the cultures positive in men when TM medium was used compared with 79.1% (68/86) and 78.1% (57/73) of the cultures respectively when MNYC medium A was used.

MIC determinations

These were carried out on isolates from nine of the 40 patients positive only on MNYC medium A and 14 of the 85 patients positive on both TM and MNYC medium A. The MICs of the nine strains isolated only on MNYC medium A were: vancomycin, 8 µg/ml (three strains), 16 µg/ml (five strains), and >32 µg/ml (one strain); lincomycin, 8 µg/ml (two strains), 16 µg/ml (six strains), and >32 µg/ml (one strain). The MICs of the 14 strains positive on

both media were: vancomycin, 8 µg/ml (two strains), 16 µg/ml (seven strains), and >32 µg/ml (five strains); lincomycin, 8 µg/ml (four strains), 16 µg/ml (seven strains), and >32 µg/ml (three strains).

Overgrowth by Proteus spp.

Using TM medium 2.0% of all sites examined in women and 0.5% of sites in men were overgrown with *Proteus* spp. compared with 0.5% and 0.17% of sites respectively when MNYC medium A was used.

Growth of unwanted micro-organisms

This was more pronounced in rectal cultures plated on MNYC medium A than on TM medium. In order to lessen contamination the lincomycin concentration was increased to 1.0 µg/ml for the second part of the study. Because of the expense of horse blood, human blood was also tested in parallel.

STUDIES WITH MNYC MEDIUM B AND MNYC MEDIUM C

MNYC medium B contained 10% horse blood and MNYC medium C 10% human blood: both contained lincomycin (1.0 µg/ml).

Women

Of the 287 female patients examined, 56 gave positive cultures on both media. One additional patient was detected by culture on MNYC medium B.

Men

Of the 417 male patients examined 67 gave positive cultures on both media. One additional patient was detected by culture on MNYC medium B.

Growth of unwanted micro-organisms seemed to be less pronounced with lincomycin (1.0 µg/ml) in the medium. However, this was not a direct comparison with the same medium containing lincomycin (0.5 µg/ml).

Discussion

MNYC medium substantially improved the efficiency and rapidity of the cultural diagnosis of gonorrhoea. In men 96.1% of cases of gonorrhoea were detected by MNYC culture compared with only 77.8% by TM; the corresponding figures for women with gonorrhoea were 100% and 69.0% respectively.

Conventional TM medium has been shown to give poor results in other trials. Willcox and John (1976) found that 91.2% of 102 male patients with positive smears gave positive cultures using the Ames detection kit which contains modified TM

medium, compared with 60.8% positive cultures using conventional TM medium: Ames detection kit or 'Microcult-GC' contains lincomycin, colistin, amphotericin B, and trimethoprim.

The better results with MNYC medium could be because of its superior nutritional value, the replacement of vancomycin with lincomycin, or a combination of these factors. The more rapid growth with MNYC medium demonstrates the improved nutritional state of MNYC medium: after 24 hours of incubation gonococcal colonies were present in 78.6% (125/159) of specimens positive on MNYC medium compared with only 47.3% (53/112) of specimens positive on TM medium. When the inoculum from the patient is low the improved nutrition provided by MNYC medium may be critical in allowing gonococcal growth.

Since none of the strains isolated only on MNYC medium was sensitive to vancomycin at the concentration present in TM medium (4.0 µg/ml) there is no direct support for vancomycin sensitivity as a cause of culture failure with TM medium. Nevertheless, the safety margin with lincomycin is much greater (MIC usually 16 times the concentration present in the medium) than with vancomycin (MIC usually two to four times greater than the corresponding medium concentration). This safety margin combined with the better nutritional value of MNYC medium may be particularly important in the case of small inocula.

Ødegaard *et al.* (1975) found that 'chocolate' (heated blood) agar medium containing lincomycin (0.5 µg/ml) in place of vancomycin (3.0 µg/ml) increased the number of samples positive for gonococci by 7%, and the number of patients with gonococcal infection by 4%; MICs were not reported in this study. These workers also found that the growth of unwanted micro-organisms was more pronounced on the medium containing lincomycin (0.5 µg/ml) in place of vancomycin (3.0 µg/ml).

Trimethoprim lactate did not completely prevent overgrowth by *Proteus* spp. but it reduced the problem considerably. It did not appear to have any inhibitory effect on gonococci since no isolates were obtained only on TM medium lacking trimethoprim.

MNYC medium was not compared directly with the NYC medium described by Faur *et al.* (1973a, b) and it is therefore impossible to assess the performance of the modified medium with the original. The comparison with our conventional TM medium and the very low proportion of smear-positive culture negative patients using MNYC medium suggests that the modified medium performs extremely well. The modified medium is much

simpler to prepare than the original since it uses a commercially available base (Difco gonococcal base). Faur *et al.* (1973a) reported that better results were obtained when the proteose peptone-cron starch agar buffered base was prepared from basic ingredients although quantitative data were not presented. The other major simplification in the MNYC medium is the use of completely lysed whole blood in place of fresh plasma and haemoglobin solution.

Faur *et al.* (1976) stated that if horse blood was not available cow blood could be substituted in MNYC medium, but that sheep blood was unsuitable: 92 of 522 cultures for *N. gonorrhoeae* were positive on both NYC media supplemented with either horse blood or cow blood, but only 85 positive cultures were obtained using sheep blood. Owing to the expense of horse blood and the small difference in the yield of positive cultures when human blood is used, this is considered to be an acceptable alternative to horse blood for the cultural diagnosis of gonorrhoea.

As a result of these findings, MNYC medium containing 10% human blood and lincomycin (1.0 µg/ml) was introduced in February 1977 for the routine isolation of gonococci.

I thank Dr D. H. H. Robertson and clinical staff of the Département of Venereology, Edinburgh Royal Infirmary for their patience during the course

of the trial. Thanks are also extended to the technical staff of the diagnostic laboratory for their expert assistance and to Professor J. C. Collee and Professor B. P. Marmion for their helpful advice during the preparation of the paper.

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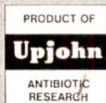
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Isolation of *Neisseria meningitidis* and *Neisseria catarrhalis* from the genitourinary tract and anal canal

CAROLINE BLACKWELL, HUGH YOUNG, AND SHEILA S. R. BAIN*

From the Department of Bacteriology, University of Edinburgh, and the University Department of Venereology,* Edinburgh Royal Infirmary

SUMMARY During a 12-month period 285 isolates of *Neisseria* species, other than *Neisseria gonorrhoeae*, were cultured from patients attending the Department of Venereology, Royal Infirmary, Edinburgh. There were eight patients in whom genitourinary or rectal isolates of *Neisseria meningitidis* or *Neisseria catarrhalis* were found. Differences between data from our series and from previous reports are discussed with particular regard to sites routinely cultured in female patients. We also report *in vitro* inhibition of *N. gonorrhoeae* by an isolate of *N. meningitidis* cultured from the cervix of a patient.

Introduction

Neisseria species other than *Neisseria gonorrhoeae* have been found to cause symptomatic infections of the genitourinary tract and the anal canal. A report of an increase in incidence of isolation as well as a brief review of the literature on *Neisseria meningitidis* infections of these sites has been recently published (Givan *et al.*, 1977). In the present study we isolated 285 non-gonococcal species of *Neisseria*. Among these there were four *N. meningitidis* isolates and five *Neisseria catarrhalis* isolates from genitourinary or rectal sites. Our findings for *N. meningitidis* differ from those previously reported—in particular, the number of male patients in the series from whom the organism was isolated, overt signs of infection, autoinoculation from the nasopharynx as a source of the organism. The possibility of inhibitory substances produced by *N. meningitidis* preventing infection by *N. gonorrhoeae* in one patient is discussed.

Materials and methods

PATIENTS

The patients in this study attended the Department of Venereology, Edinburgh Royal Infirmary between 1 July 1976 and 30 June 1977. During this

period there were approximately 2000 women and 2500 men.

In men, if a urethral discharge was present, or if there was presumptive evidence of contact with gonorrhoea, a Gram-stained film of urethral discharge was examined microscopically and material inoculated directly on to culture plates. In the case of homosexuals, material from the urethra, rectum and pharynx was cultured routinely, and if negative on the first occasion, repeated twice at weekly intervals.

In women, Gram-stained films of material from the urethra and cervix, and cultures from the urethra, cervix, rectum and, if indicated, from the pharynx were taken. Generally, if the first cultures were negative for *N. gonorrhoeae*, these were repeated twice at weekly intervals.

CULTURE AND IDENTIFICATION

During the first six months of the study cultures were made on Thayer-Martin medium containing the antibiotics vancomycin (4.0 µg/ml), colistin (6.0 µg/ml), and nystatin (10.0 µg/ml). During 1977, cultures were made on modified New York City Medium (Young, 1978) containing the antibiotics lincomycin (1.0 µg/ml), colistin (6.0 µg/ml), amphotericin B (1.0 µg/ml), and trimethoprim lactate (6.5 µg/ml).

Cultures were incubated at 36°C in a carbon dioxide enriched (10%) atmosphere. After incubation for 24 hours, plates were examined and any suspect colonies tested by the oxidase reaction and Gram's stain. Oxidase positive Gram-negative diplococci

Address for reprints: C. Blackwell, Department of Bacteriology, University of Edinburgh, Medical School, Teviot Place, Edinburgh EH8 9AG

Received for publication 22 July 1977

were identified by the rapid carbohydrate utilisation test and direct immunofluorescence using Difco fluorescein-labelled anti-gonococcal conjugate as described by Young *et al.* (1976). Negative cultures were re-examined after 48 hours of incubation.

Demonstration of the inhibitory substance from *N. meningitidis* was by means of the soft agar method for bacteriocin production (Fredericq, 1957).

Results

During the 12-month period from 1 July 1976 to 30 June 1977 approximately 4500 patients attended the Department of Venereology, Royal Infirmary, Edinburgh. There were 350 specimens of *N. gonorrhoeae* isolated from the 2000 female patients and 480 specimens isolated from the 2500 male patients.

The *Neisseria* isolates, other than *N. gonorrhoeae*, from these patients are summarised in Table 1.

Table 1 Isolation of *Neisseria* species other than *N. gonorrhoeae* over a 12-month period

<i>Neisseria</i>	Total no. of isolates	Site of isolation		
		Throat	Genito-urinary tract	Anal canal
<i>N. meningitidis</i>	250	246	2	2
<i>N. lactamica</i>	23	23	0	0
<i>N. catarrhalis</i>	7	2	1	4
<i>N. flava</i>	3	3	0	0
<i>N. perflava</i>	2	2	0	0

N. meningitidis was the species most often isolated (250); among these there were two from the anal canal and two from the genitourinary tract. We found *N. lactamica* (23), *N. flava* (3), and *N. perflava* (2) only in throat cultures. Of the seven *N. catarrhalis*, two were from throat cultures, one was from the genitourinary tract, and four from the anal canal.

There were four patients from whom *N. meningi-*

tidis was isolated. The information available for each patient is summarised in Table 2. The only additional systemic complaints from Patient 1 were a loss in weight of 7 lb (3.175 kg), dysuria, and frequency of micturition. *N. gonorrhoeae* were not isolated at this time nor on the three subsequent sets of cultures. She had had a regular consort for the past two years and the only previous history of a sexually transmitted disease (STD) was warts in 1975 and genital candidosis in 1974. A second rectal isolate was from Patient 2 who had attended the clinic four months previously, at which time *N. meningitidis* was isolated from the throat. The only systemic complaint was eczema on face, trunk, and limbs and there was no previous history of STD. Patient 3, a bisexual male with no previous history of STD, had attended the clinic two months earlier. At that time *N. meningitidis* was isolated from the throat but no *Neisseria* had been found in cultures of rectum or urethra.

The only patient in the series from which we isolated *N. meningitidis* and, later, *N. gonorrhoeae* was Patient 4. She was seen in the clinic as a gonorrhoea contact; her husband had become infected from an extramarital consort. Microscopical examination of material from the cervix revealed large numbers of polymorphonuclear leucocytes with intracellular Gram-negative diplococci. She was treated with co-trimoxazole as there was a possibility of penicillin allergy. The culture of cervical material grew only *N. meningitidis* and no gonococci were isolated from any other site. A month later gonococci were cultured from the cervix and she was treated with another course of co-trimoxazole. Disc sensitivity tests revealed both the previously isolated meningococcus and the gonococcus to be sensitive to trimethoprim-sulphamethoxazole (disc containing 23.5 µg sulphamethoxazole, 1.5 µg trimethoprim). At the time it was serotyped, the meningococcus was

Table 2 Isolation of *N. meningitidis*

Patient	Sex	Site of isolation	Throat culture	Serotype	Reason for attending STD clinic	Diagnosis, treatment, and follow-up
1	F	Rectum	—	B	Warts	Cautery and liquid nitrogen for warts; 3 subsequent sets of cultures negative
2	F	Rectum	Not done	B	Frequency of micturition; warts	Podophyllin and cautery for warts; no follow-up cultures subsequent to isolation of <i>N. meningitidis</i> from anal canal
3	M	Urethra	Not done	NT	Syphilis contact	No treatment; no follow-up cultures
4	F	Cervix	—	NT	Gonorrhoea contact	Asymptomatic gonorrhoea (only <i>N. meningitidis</i> cultured); co-trimoxazole 5 days; 2 subsequent sets of cultures negative; <i>N. gonorrhoeae</i> cultured 3 times during next 3 months

— = no *Neisseria* isolated

NT = non sero-typable *N. meningitidis*

further tested and found to grow on 0.1 µg/ml sulfadiazine but not on 1 µg/ml. She denied any sexual contact with her husband during this period. The soft agar method for demonstrating sensitivity to bacteriocins showed the meningococcus elaborated a substance which greatly inhibited the gonococci from Patient 4 and several other *N. gonorrhoeae* isolates from different sources. It was later discovered that the gonococcal infection was probably not a treatment failure or inhibition by the meningococcal substance but infection from an extramarital contact. There was no systemic upset in Patient 4, but her extramarital contact and his wife developed systemic infections and arthritis.

There were four female patients from whom *N. catarrhalis* was isolated (Table 3). None of these had any systemic upset and no gonococci were cultured at follow-up visits.

Discussion

Givan *et al.* (1977) have reviewed case reports of isolation of *N. meningitidis* from the genitourinary tract and anal canal. Our data from the past 12 months provide a somewhat different view. These differences may be owing to routine culturing of urethra, cervix, and rectum of all female patients. Previous reports mention only cervical cultures of women, with additional blood or joint cultures from two systemic infections (Keys *et al.*, 1971; Morgan *et al.*, 1976). Other series contain a predominance of men, 58 of the 84 patients in the literature; in our study three of the four patients from whom we isolated *N. meningitidis* and all of the patients from whom we isolated *N. catarrhalis* were women.

There were no overt symptoms of these meningococci causing infection in any of the patients, again in contrast to the findings of others. The main reason for attending the Department of Venereology was genital warts (four out of eight patients). Three were either syphilis or gonorrhoea contacts, and the fourth was referred for follow-up of a gonococcal

infection. There was no evidence of rectal infections with *N. meningitidis* associated with systemic infection. Currently there is no evidence for treating rectal carriers, but follow-up cultures to monitor any spread to the urethra or the cervix may be of value.

It has been suggested (Givan *et al.*, 1977) that there may be autoinoculation of these areas from the nasopharynx. In their series all six of the rectal carriers from whom there were throat cultures had meningococcus in the pharynx. In our series, none of the patients from whom throat cultures were taken was carrying *N. meningitidis* in the nasopharynx. It is also interesting to note that meningococci were not isolated from either contact of Patient 4.

The significance of the inhibitory effect of *N. meningitidis* from Patient 4 on *N. gonorrhoeae* is difficult to assess. A similar instance of apparent protection has been reported for a male patient with asymptomatic meningococcal urethritis (Volk and Kraus, 1973). The role of this inhibitory effect in preventing infection from her initial exposure is not clear. The meningococcus would probably have been eliminated by the first co-trimoxazole treatment before her second exposure and subsequent infection with *N. gonorrhoeae*.

These observations underline the necessity of correct and full identification of all neisserial isolates, even when a selective medium is used. These non-gonococcal *Neisseria* represent approximately 2% of the anogenital isolates in women and 0.2% of those from men.

This work is part of a project supported by a grant from the Scottish Home and Health Department, Medical Research Committee.

Serotyping of meningococci and sulphadiazine sensitivity testing was kindly carried out by Dr R. J. Fallon, Department of Laboratory Medicine, Ruchill Hospital, Glasgow.

Table 3 Isolation of *N. catarrhalis*

Patient	Sex	Site of isolation	Throat culture	Reason for attending STD clinic	Diagnosis, treatment, and follow-up
5	F	Rectum	—	Gonorrhoea contact	No treatment; 3 subsequent sets of cultures negative
6	F	Rectum	Not done	Warts	Podophyllin for warts; 3 subsequent sets of cultures negative
7	F	Rectum	Not done	Follow-up treatment for gonorrhoea	Non-gonococcal vaginal discharge; nystatin for discharge
8	F	Urethra Rectum	Not done	Warts	Podophyllin for warts; 4 subsequent sets of cultures negative

— no *Neisseria* isolated

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Chlamydial infection of the urethra in men

H. M. PERROUD AND K. MIEDZYPBRODZKA

From the Department of Dermato-Venereology, University of Lausanne, Switzerland

SUMMARY *Chlamydia trachomatis* was isolated from the urethra of 124 (52%) of 238 men with non-gonococcal urethritis (NGU). Repeat isolation attempts in 155 of these patients were successful in eight men in whom results had been negative on the initial visit, but they were unsuccessful in eight men who initially had had positive cultures. We must assume that with our present isolation techniques we are missing, at any single visit, at least 9% of chlamydial infections. *C. trachomatis* was also found in 32 (23%) of 139 men with gonorrhoea. Positive cultures were obtained from 15 (79%) of 19 men, who later developed post-gonococcal urethritis (PGU). Thiamphenicol, used for the treatment of gonorrhoea, was shown to have very little effect on *C. trachomatis*, which could still be recovered after treatment in 76% of the patients who initially had had a combined infection. The typing of 35 genital isolates by micro-immunofluorescence confirms the previously reported distribution of chlamydial serotypes. In this study a social profile is given of our patients with urethritis and a comparison is made of the duration of symptoms and the nature of discharge in men with gonococcal, chlamydial, and non-specific urethritis. We were able to show a clear difference in clinical symptoms in men with gonorrhoea and NGU, taken as a whole, but found only a slight difference between men with chlamydial and non-specific urethritis.

Introduction

The incidence of non-gonococcal urethritis (NGU) has risen more rapidly than that of gonorrhoea in England (Department of Health and Social Security, 1976), Sweden (Juhlin, 1975), and the USA (McChesney *et al.*, 1973) and it is now recognised as the most common sexually transmitted disease. Although no statistics are available as NGU is not reported, data from the Department of Dermato-Venereology in Lausanne suggest that this trend also applies to Switzerland (Perroud, 1977). NGU accounted for 55% of all our cases of urethritis in 1976. *Chlamydia trachomatis* has been incriminated as one of the most common sexually transmitted genital pathogens, and is reported to be associated with approximately half of all cases of NGU. Simplified cell culture techniques can now isolate *C. trachomatis* and make it possible to carry out larger epidemiological studies. We hoped to determine the incidence of chlamydial infections in

patients presenting with urethritis and to define more clearly their clinical characteristics in comparison with gonococcal and non-specific urethritis (NSU).

Materials and methods

SELECTION OF CASES AND DIAGNOSTIC CRITERIA

The patients, all men, were examined between March 1975 and April 1977 in the Department of Dermato-Venereology of the University Hospital in Lausanne. All had presented with the complaint of urethral discharge with or without dysuria. A standard medical history was taken for each patient concerning symptoms and findings, previous genital infections, and sexual contacts. The clinical assessment included collecting secretions for bacteriological examination after gently stripping the anterior urethra. When no exudate could be obtained by this method, prostatic massage was performed.

GONORRHOEA

Urethral smears showing Gram-negative intracellular diplococci were considered as presumptive

Address for reprints: H. M. Perroud, Department of Dermato-Venereology, CHUV, 1010 Lausanne, Switzerland

Received for publication 8 July 1977

evidence and confirmed in all cases by a positive culture for *Neisseria gonorrhoeae*. Cultures were made on Thayer Martin selective culture medium (Baltimore Biological Laboratories).

NON-GONOCOCCAL URETHRITIS

Only cases with visible discharge, either spontaneously or after gentle urethral massage, and more than 20 leucocytes on at least three fields examined at $\times 400$ magnification were included. Gonorrhoea was excluded by a negative smear result and by at least one negative culture; two culture attempts were performed in most cases. *Trichomonas vaginalis* infections were excluded by wet films.

POST-GONOCOCCAL URETHRITIS

Post-gonococcal urethritis (PGU) was diagnosed if the patient still had a discharge seven or more days after treatment for gonorrhoea, or if a discharge appeared on gentle urethral massage, smears and cultures were negative for gonococci and trichomonads, but the microscopical criteria for NGU were fulfilled.

CONTROL GROUP

Men included in this group had neither a history nor symptoms of urethritis and matched the groups with gonococcal and non-gonococcal infections in regard to age, marital state, percentage of homosexuals, and number of partners. Twelve of the controls were non-infected contacts of women with gonorrhoea, trichomoniasis, or candidosis; 14 had genital conditions other than urethritis, 10 were students or staff, and four men requested examination but nothing was found.

CRITERIA FOR EXCLUSION

Patients who had received antibiotics during the two weeks before the initial visit were not included in the study. A few patients with gonorrhoea and NGU had to be excluded because inoculation of the cultures was delayed for more than 24 hours, or because contamination of the cell cultures rendered the isolation of *C. trachomatis* impossible. No other criteria were used to divide the patients into groups.

ISOLATION OF *C. TRACHOMATIS*

Collection of specimens

The method of Reeve *et al.* (1975) was used to collect specimens and isolate *C. trachomatis*. Urethral specimens were collected by inserting a calcium-alginate-tipped wire swab 3-4 cm into the urethra. The tip was then cut into a vial containing 2 ml of transport medium. This transport medium consisted of growth medium (see below) supplemented with glucose 1% by vol. of stock (3 mol/l)

and amphotericin B 2.5 $\mu\text{g/ml}$. The collected specimens were kept at a temperature of at least 4°C and isolation was attempted within 24 hours.

Cell cultures

McCoy cells were grown in Eagle's minimal essential medium supplemented with heat inactivated fetal calf serum 10% by vol., glutamine 1% by vol. of stock (30 mg/ml), and vitamins (Flow lab. 100 $\times$ concentrate) to which was added gentamicin 0.1 mg/ml. When confluent, the cells were trypsinised and suspended at 1×10^6 cells/ml of growth medium to which was added 5-iodo-2'-deoxyuridine (IUDR) 25 $\mu\text{g/ml}$. One millilitre of this suspension was placed in each 10 ml flat-bottomed vial containing a 12 mm diameter coverslip. After incubation at 35°C for three days the monolayers were ready for chlamydial isolation and were used for up to seven days after seeding.

ISOLATION OF *C. TRACHOMATIS*

To inoculate the specimens, the growth medium was removed from each of two vials with confluent monolayers and replaced by half of the transport medium (1 ml). The vials were then centrifuged in a Mistral 6 L centrifuge at 2700 g for an hour at 35°C. They were then incubated at 35°C for two days when the medium from one was removed, the monolayers fixed with methanol for 10 minutes, and then stained with Lugol's iodine for five minutes. The coverslips were removed from the vials, air-dried, and mounted in immersion oil. They were then examined at $\times 400$ magnification for typical chlamydial cytoplasmic inclusions. The duplicate vial was kept for further passage.

CHLAMYDIAL STRAIN TYPING

For strain typing the four-day mouse antisera method of Wang *et al.* (1973) was used. The sera were then typed according to the micro-immunofluorescence (MIF) test as described by Wang (1971). The antigens consisted of abundantly infected yolk sac suspensions. The original typed strains for the preparation of antigen were kindly supplied by Dr J. Trehanne of the University of London and Dr C. Dawson of the WHO Chlamydia Reference Center in San Francisco.

ISOLATION OF *N. GONORRHOEAE*

Thayer-Martin selective culture medium was inoculated beside the patient and immediately incubated in a candle extinction jar at 36°C. Identification of morphologically typical, oxidase-positive colonies was carried out by using the delayed fluorescent antibody technique with a commercially available antiserum (Difco).

Results

ISOLATION OF *C. TRACHOMATIS* IN MEN WITH GONOCOCCAL AND NON-GONOCOCCAL URETHRITIS

The χ^2 test and analysis of variance followed by the Scheffé method were used to analyse the results.

Altogether 139 men with gonococcal urethritis were examined for the presence of *C. trachomatis*. Positive cultures for this organism were obtained from 32 (23%) of them (Table 1).

Table 1 Incidence of *C. trachomatis* infections in men with NGU and gonorrhoea

	No.	Chlamydia-positive	
		No.	%
NGU	238	124	52
Gonorrhoea	139	32	23
Control group	40	1	2.5

All these patients with gonorrhoea were treated with a single dose of 2.5 g thiamphenicol by mouth and cure was confirmed in 93% by a negative smear and culture on a follow-up visit one week after treatment. Nineteen men whose gonorrhoea had been treated still had a urethral discharge at the follow-up visit although they had a negative culture for *N. gonorrhoeae*. These patients' condition fulfilled the criteria outlined and was diagnosed as PGU. Fifteen (79%) of them initially harboured *C. trachomatis*, which could still be recovered at the time PGU was diagnosed.

We also followed-up 10 patients, who initially had a combined infection but who did not develop any sign of PGU after treatment. At the follow-up visit five (50%) were still positive for *C. trachomatis*.

The effect of thiamphenicol as a single dose of 2.5 g on a genital infection with *C. trachomatis* can be assessed by the isolation rate after treatment in men with a proved infection. We obtained a negative culture result at the follow-up visit in seven men (24%), whereas 22 (76%) of a total of 29 patients still harboured *Chlamydia* one week after treatment.

The overall isolation rate of *C. trachomatis* in the group of 238 unselected patients with NGU was 52% (Table 1), three of these patients presented with urethritis associated with epididymitis and one patient had Reiter's disease. In 155 men with NGU specimens for *C. trachomatis* cultures were taken on their first visit and again about three days later on their second visit before treatment was initiated. Table 2 shows the recovery of *C. trachomatis* at each visit. We were able to re-examine 64 out of 116 men who had positive cultures on the initial visit. In eight (11%) of these patients the repeat culture, proved negative. Of the 122 men, who

initially had a negative culture 91 were re-examined and eight (9%) were found to be positive on that occasion.

Table 2 Recovery of *C. trachomatis* in patients with NGU on two consecutive visits

Visit	No. of patients examined	Chlamydia-positive		Chlamydia-negative	
		No.	%	No.	%
First	238	116	49	122	51
Second	64	56	89	8	11
Chlamydia-negative	91	8	9	83	91

Average interval between the first and second visit: 2.7 days

The duration of symptoms recorded at the initial visit in these eight patients was: 2, 4, 5, 8, 8, 14, 16, and 16 days.

In the group of 40 controls, *C. trachomatis* was found in one of them (isolation rate 2.5%).

SEROTYPES OF CHLAMYDIAL ISOLATES

We were able to establish the serotype according to the classification of Wang *et al.* (1973) from 35 chlamydial isolates recovered from 29 men and six women. The results of micro-IF typing are shown in Table 3.

Table 3 Results of serotyping chlamydial isolates by micro-IF test

Total no. of isolates	Serotype			
	C/J	D/E	F/G	K
35	1 (2.8%)	18 (51.4%)	10 (28.5%)	6 (17.1%)

SOCIAL PROFILE AND CLINICAL MANIFESTATIONS OF MEN WITH GONORRHOEA, CHLAMYDIAL URETHRITIS, AND NSU

We compared the social profile and symptoms of men with gonorrhoea, *Chlamydia*-positive urethritis, and NSU. The profile of men with chlamydial urethritis by age (mean: 28 years), marital state (76% single, 24% married), and percentage of homosexuals (5%), was similar to the one established for gonorrhoea and NSU. The degree of promiscuity in the three groups, expressed by the number of recent sexual partners was also similar as shown in Table 4.

Table 4 Number of recent sexual partners of men with gonococcal, chlamydial, and non-specific urethritis

No. of partners	Gonorrhoea (%)	Chlamydial urethritis (%)	NSU (%)
One	36	41	48
Two	27	32	29
Three or more	37	27	22
Total no. of patients	90	91	85

A history of previous episodes of urethritis, without a more precise definition, was obtained from 63% of patients with chlamydial urethritis and 60% with NSU, whereas only 41% of the men with gonorrhoea indicated previous genitourinary symptoms. In Table 5 the duration of urethral discharge is compared for the three groups. Most patients had symptoms for less than 14 days, but in each group there were several patients who had had symptoms for up to a year which made it necessary for a more accurate comparison to establish two groups (≤ 14 days and > 14 days).

Table 5 Comparison of clinical characteristics of gonococcal, chlamydial, and non-specific urethritis

Symptoms	Gonococcal urethritis		Chlamydial urethritis		Non-specific urethritis	
	No.	%	No.	%	No.	%
Duration of discharge						
<14 days	90		70		68	75
Duration (mean)		3.8		6.1		4.8
>14 days	10		32		32	18
Duration (mean)		28		33		75
Dysuria						
Present	62		90	68	63	60
Absent	7		10	39	37	27
Nature of discharge						
Purulent	60		62	10	9	19
Mucopurulent	34		36	53	50	52
Mucoid	2		2	43	41	29
Absent	3		3	8	7	11

Dysuria was found significantly more often in patients with gonorrhoea (90%) compared with *Chlamydia*-positive (63%) and *Chlamydia*-negative (69%) NGU ($\chi^2=15.17$; $P<0.001$). The incidence of this symptom in the two groups with NGU was similar. The urethral discharge was different in men with gonorrhoea, being mostly purulent (62%) and mucopurulent (36%) compared with NGU (*Chlamydia*-positive and *Chlamydia*-negative), which tended to be more mucoid and mucopurulent ($\chi^2=88.86$; $P<0.001$). Table 5 shows the mean duration of discharge found in each group. Only the patients who had had a discharge for less than 14 days could be compared by statistical methods. The difference between the duration of discharge in gonorrhoea and NGU (*Chlamydia*-positive and *Chlamydia*-negative) was highly significant ($P<0.01$). On the other hand, the values obtained in chlamydial urethritis and NSU did not differ significantly ($P=0.06$). In each group a few patients showed no urethral exudate on the first examination, although all of them gave a history of discharge during the days preceding examination.

Discussion

We found a *C. trachomatis* isolation rate of 52% in our 238 patients with NGU. This incidence compares well with the recovery rates previously reported in this condition. Holmes *et al.* (1975) reported 42%, Oriel *et al.* (1976) reported 48%, Alani *et al.* (1977) and Schachter *et al.* (1975) obtained even higher isolation rates—67% and 57%, by only selecting cases of NGU with severe urethral discharge, which may correlate with a higher concentration of infectious elementary bodies in the exudate. An important question is the diagnostic yield of a single isolation attempt for *C. trachomatis*. To clarify this problem we repeated sampling, whenever possible, before initiating treatment. Two isolation attempts on separate visits at a mean interval of 2.7 days were performed in 155 patients with NGU. On the second examination we missed 11% of the chlamydial infections in the group of patients with positive cultures on the first visit. On the other hand, 9% of men with negative results to their first cultures proved positive on the second attempt. Thus we must assume, that with our present sampling and isolation techniques and no delay exceeding 24 hours before inoculation, we are missing at least 9% of chlamydial infections at any visit. It can be assumed that a third isolation attempt would provide an additional number of positive results. Some workers (Richmond *et al.*, 1972; Oriel *et al.*, 1976; Alani *et al.*, 1977) found a highly significant relationship between the duration of symptoms and the rate of chlamydial isolation. This relationship could not be substantiated in our patients. The difference in duration of symptoms and time of sampling for cultures in our patients with *Chlamydia*-positive and *Chlamydia*-negative NGU was statistically not significant. Furthermore, in 91 out of 122 men with *Chlamydia*-negative NGU a second isolation attempt was performed, on an average of 2.7 days later. In only eight (9%) of these men was the culture positive, of whom only three had had symptoms for less than six days. These findings indicate that in most men with NGU, chlamydial infections can be diagnosed within the first days after the onset of symptoms, and that it is unlikely that we are missing this organism in the group of men with NGU because sampling for culture is performed too early in the course of infection.

C. trachomatis was also recovered in 23% of patients with gonorrhoea. This rate is similar to the results reported in previous studies where 25% (Oriel *et al.*, 1976) and 32% (Richmond *et al.*, 1972) of men with gonorrhoea were also infected with *C. trachomatis*. As all our patients, with

gonorrhoea were treated by a single dose of 2.5 g of thiamphenicol, we were able to obtain some information on the effect of this antibiotic on *C. trachomatis*. It appears that thiamphenicol, at the dosage used, has no effect or only a marginal one on *C. trachomatis*, as this could still be demonstrated at the follow-up visit in 76% of patients who had had a positive culture before treatment. Furthermore, the simultaneous infection with *C. trachomatis* and *N. gonorrhoeae* led to the development of PGU in 68% of these patients; an incidence of PGU of only 4.7% was observed in men without concomitant chlamydial infection. As Holmes *et al.* (1975) and Oriel *et al.* (1975) have indicated, this striking correlation between the presence of *C. trachomatis* and the development of PGU provides strong evidence in support of the pathogenicity of this agent in the urethra. Further evidence for this hypothesis is that *C. trachomatis* can be found only rarely in asymptomatic men. Schachter *et al.* (1975) reported the highest percentage of isolates (7%) in a control group. We were able to discover only a single case in our control group of 40 men.

The results of immunotyping the chlamydial isolates are similar to the distribution of serotypes observed in England (Treharne *et al.*, 1972) and in the USA (Holmes *et al.*, 1975). The most prevalent serotypes in the genital tract belong to the group D/E, which we found in 51% of our typed isolates. The groups F/G and K were also well represented, but C/J was found only once. We were not able to detect any isolates belonging to types H and I, which have also been recovered from the genital tract.

The social profile of men with gonorrhoea and NGU was very similar in our groups. An equivalent percentage (63% and 60%) of men with chlamydial urethritis and NSU indicated that they had had previous episodes of genitourinary symptoms. This contrasts with the findings of Alani *et al.* (1977), who obtained higher isolation rates in men with no previous episodes of urethritis. Our data also showed that men with NGU and men with gonorrhoea generally present with a different history and different clinical manifestations, which may be used with the usual bacteriological techniques, to differentiate the two entities (Jacobs and Kraus, 1975). Unfortunately, these differences in symptoms do not appear as clearly when *Chlamydia*-positive and *Chlamydia*-negative NGU is compared. The duration of discharge before attendance was shortest in gonococcal urethritis (3.8 days), intermediate for NSU (4.8 days), and longest in chlamydial urethritis (6.1 days). However, the difference between the two groups with NGU is not significant. Dysuria was present in 90% of men with gonorrhoea, in 69% of men with NSU, and it was only slightly less

common in men with chlamydial urethritis (63%).

When the nature of the urethral discharge was compared, it appeared that gonorrhoea most often caused a purulent discharge, whereas chlamydial urethritis was generally characterised by a watery mucoid or mucopurulent discharge. The longer duration of symptoms, the presence of dysuria in little more than half of the patients, and the mucoid or mucopurulent urethral exudate confirm that the chlamydial infection of the male urethra generally runs a subacute and mildly symptomatic course, in contrast with a gonococcal infection, which causes a more acute and severe inflammation. The presence or absence of *C. trachomatis* in NGU, however, cannot be suspected on clinical grounds alone, as neither history nor clinical signs seems to differ sufficiently.

We are grateful to Dr G. Van-Melle, Division d'Informatique, CHUV, Lausanne, for carrying out the statistical analysis.

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A trial of adenine arabinoside in genital herpes

A. L. HILTON, T. E. C. BUSHELL, D. WALLER, AND JOY BLIGHT

From the Departments of Venereology and Virology, Bristol Royal Infirmary

SUMMARY Twenty-four episodes of genital herpes in 22 men, all confirmed by virus isolation, were studied in a double blind trial. They were treated either with 3% adenine arabinoside (Ara-A) in petrolatum ointment base or with the base alone applied four times daily for one week. The lesions were counted and sketched on days 0, 2, and 7. There was no demonstrable advantage to be gained from the use of Ara-A ointment. The results of the Papanicolaou smears and virus isolation agreed in the diagnosis of 75% of cases. The presence of continuing lesions or fresh ones in some patients after the acute phase of the initial or recurrent attack of herpes necessitates the man taking precautions on resuming sexual intercourse.

Introduction

There is a continuing need to study antiviral drugs that may inhibit or kill genital herpes simplex virus (HSV) Type 2. A primary infection in the male is usually painful and will last between two and three weeks or longer, whereas a recurrent attack is more likely to be relatively painless and transient, or to persist for less than two weeks. The female partner on the other hand can occasionally develop more extensive and painful lesions of the genitalia and if the cervix is affected there can be serious sequelae. Most experimental drugs so far investigated are purine and pyrimidine derivatives. Adenine arabinoside (vidarabine, Vira-A, Ara-A) is a purine nucleoside (9-B-D-arabinofuranosyladenine) which has been shown to be active against deoxyribonucleic acid (DNA) viruses, including members of the herpes group (Schabel, 1968). Its therapeutic effect in life-threatening DNA viral infections (Keeney and Buchanan, 1975), including those in immunosuppressed patients (Ch'ien *et al.*, 1976), is being assessed and if drug toxicity occurs it appears to be dose related (Ross *et al.*, 1976). Topical application in the treatment of herpetic infections of the eye has had encouraging results (Pavan-Langston *et al.*, 1973; Coster *et al.*, 1976).

Three per cent Ara-A in petrolatum ointment base was made available for evaluation in the treatment of genital herpes in the male. As all herpetic

infections are subject to latency, chronicity and recurrence, a double blind randomised comparison with a petrolatum placebo was used, each preparation being administered for seven days.

Patients and methods

All patients were seen in the Department of Venereology, Bristol Royal Infirmary between August 1974 and October 1975 and only those thought likely to return for follow-up visits were included. Patients with other sexually transmitted diseases were excluded as were those with severe secondary infection, so there were few patients receiving other concurrent forms of treatment. Each patient was questioned about any history of genital ulcers, and on the duration of symptoms, including that of pain, in the present attack. At each visit the number and distribution of lesions, old or fresh, were sketched. After treatment it was sometimes necessary to estimate the number of lesions if these were old and had coalesced. The old lesions were described as having 'not changed', 'dried', 'scabbed', or 'healed'; old lesions were said to have healed when there was no visible abnormality or only a residual slight erythema. Most of the vesicles were therapeutically ruptured on the first visit so aiding later differentiation of fresh from old lesions. Routine darkground microscopical examination for *Treponema pallidum* was undertaken together with serological screening. Haematological and transaminase investigations and urine analysis were also carried out and patients were requested to return for re-investigation one month later.

Address for reprints: A. L. Hilton, Special Clinic, Level Three, Bristol Royal Infirmary Extension, Bristol BS2 8HW

Received for publication 25 July 1977

VIROLOGY

Dry swabs were used for collecting material for the culture of HSV from patients on their initial visit and on more than 80% of the subsequent attendances when ulcers or blisters were present. The cottonwool swabs were transported in 50% skimmed milk in saline (Clarke *et al.*, 1964). Each specimen was inoculated into cultures of human embryo fibroblasts and Hep 2 cells on the day of receipt, except for specimens taken after routine laboratory hours which were processed the following day. The identity of the isolates was confirmed by neutralisation with specific herpes simplex antiserum and then Types 1 and 2 were differentiated by (a) the cytopathic effect in BHK-21 cells (Smith *et al.*, 1973) and (b) the nature of the lesions produced on the chorioallantoic membrane of fertile hens' eggs when inspected at seven days (Parker and Banatvala, 1967). Papanicolaou smears were prepared from scrapings of the penile lesions with a coverslip when the patients first attended, and again from 40% of the lesions which were swabbed for virus isolation during the trial period. Each patient was supplied with either Ara-A or the vehicle ointment in a double blind randomised trial with instructions to apply the ointment four times daily for one week. No attempt was made to confirm that the ointment was regularly applied other than to request that used and unused tubes be returned at the end of treatment. After their first attendance patients were seen 48 hours later (day 2), at the end of the treatment (day 7), and at the end of the trial period (day 28).

Results

Thirty-one patients took part in the trial, contributing 34 episodes of herpes genitalis. Five patients had histories of some form of allergy, and nine had received local applications to the skin. One patient received metronidazole and another erythromycin for accompanying tonsillitis during the seven days of treatment. The clinical features and the results of investigations carried out on presentation are listed in Table 1. When efficacy of treatment was compared (Table 2), five episodes in each group were excluded either because the virus cultures were

Table 1 Positive findings at initial visit

	Treatment	
	Ara-A (total=17 episodes)	Placebo (total=17 episodes)†
Previous genital herpes	5	9
Confirmation by virus culture‡	12 (11 Type 2) (1 Type 1)	14 (all Type 2)
Papanicolaou smear positive	8*	11
Regional adenopathy	11	7
Secondary infection	7	4
Darkground illuminated		
<i>Treponema pallidum</i>	0	0*

*One patient omitted

†Three cases initially negative, positive on day 2

‡Seventeen episodes in 15 patients, one patient later treated with Ara-A

negative or because the patients were uncooperative, thus leaving 12 episodes to be assessed in each group. One patient appeared in both groups; he was first treated with the placebo and four months later with Ara-A. Another patient was seen twice in the placebo group with an interval of more than six months. Both patients had a history of previous herpes infection. The treatment groups were similar when compared for complaints of pain, duration of symptoms, and number of lesions. Ara-A was no better than the placebo ointment in healing the original ulcers or preventing fresh lesions or late recurrences (see Tables 2 and 3).

Table 3 confirms the well established facts that first attacks of herpes simplex virus infection are associated with a greater number of lesions and take longer to heal than recurrences. At the end of a week's treatment with either placebo or Ara-A, 56% of the recurrences had healed compared with only 20% of the first attacks. Fresh lesions were observed in some patients during the seven days of treatment, during the next three weeks of the trial, and in the subsequent months when patients returned to the department for further advice. HSV was isolated either on day 2 or day 7 in seven of ten cases using Ara-A and in eight of nine cases using the placebo ointment; in the remainder the ulcers had healed or were not sampled. There was reasonable correlation between the results of virus

Table 2 Response to treatment

Treatment	No. of patients	Day 0		Day 2		Day 7	
		Patients with pain	Duration of symptoms Mean no. (days)	No. of patients		No. of patients	
				Lesions healed	New lesions	Lesions healed	New lesions
Ara-A	12	9	6.6	0	2	4	2
Placebo	12*	7	4.4	0	2	4	3

*Twelve episodes in 11 patients, one patient later treated with Ara-A

Table 3 *The relationship of history of infection with results of treatment and new lesions*

Type of infection	Treatment	No. of patients	Mean total no. of lesions		No. of patients			
			Before treatment	After treatment	All lesions healed	With new lesions		
						At day 7	During treatment	After treatment
						Day 7-28	> 28 days	
Recurrence	Ara-A	2	6.5	0	2	—	—	2
	Placebo	7*	8	3.6	3	3	3	2
Initial	Ara-A	10	15.8	7.5	2	4	1	2
	Placebo	5	16.6	8.6	1	2	2	2

*Seven episodes in 6 patients, one patient later treated with Ara-A.

culture and Papanicolaou smear with agreement in 75% of 52 specimens, both being reported positive on 23 occasions and negative on 16 occasions. The cytology was positive three times when virus culture was negative and virus culture was positive 10 times when the smear was negative. The isolation of HSV was a more sensitive diagnostic technique than the smear, particularly when there were insufficient numbers of squames in which to look for the typical multinucleate giant cells.

There was no adverse reaction in any patient treated with Ara-A, but in the control group one patient reported excessive moisture of the treated area which could have been related to treatment. However, two episodes of skin rashes in the same group were thought not to be related to drugs. Haematological and transaminase results and urine analysis failed to indicate drug toxicity.

Discussion

This investigation revealed that Ara-A possesses no advantage over placebo. It did not shorten the length of an attack, prevent recurrences, or reduce the excretion of HSV in the treatment of genital herpes in the male.

Idoxuridine (IDU) was found to be insoluble in most solvents until dimethyl sulphoxide (DMSO) was used (MacCullum and Juel-Jensen, 1966). It is possible that the DMSO or another base may be identified which would allow the relatively insoluble Ara-A to penetrate the skin more effectively, but even then the virus in the nerve and sacral ganglia would presumably be left intact. Systemic treatment might be curative but the condition is not serious enough to warrant this route of administration except in rare instances.

Fresh lesions, some relatively painless and inconspicuous, were observed during the week of treatment and the next three weeks. A new female partner would have been at risk from such a patient who practised unprotected sexual intercourse unaware of his continuing infectivity. That some at least of these new lesions could be associated with

the petrolatum base was well demonstrated by Adams *et al.*, 1975. They used the same Ara-A and placebo ointments with similar results, but they also included a group of patients left untreated, none of whom developed any fresh lesions.

We are indebted to Professor David Montefiore, Dr A. E. Tinkler, and Dr David Evans for their advice in the preparation of this trial and to the staff of this department, especially Mr A. G. Martin, for their assistance. We wish to acknowledge the laboratory aid from our colleagues in the departments of biochemistry and haematology and the provision of the trial ointments by Parke-Davis.

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Circulating immune complexes in syphilitic nephropathy

A case report

J. SØLLING, K. SØLLING, K. U. JACOBSEN, S. OLSEN, AND E. FROM

From the Department of Medicine C and University Institute of Pathology, Aarhus Kommunehospital and Department of Dermato-Venerology, Marselisborg Hospital, University of Aarhus, Aarhus, Denmark

SUMMARY A case of transient nephrotic syndrome caused by secondary syphilis is described. A renal biopsy was performed revealing subepithelial hump-like electron-dense deposits and fusion of epithelial foot-processes. Complement C1q-binding-activity and anticomplementarity were demonstrated in the blood, indicating the presence of circulating immune complexes. This strongly suggests that circulating immune complexes are significant in the immunopathogenesis of syphilitic nephropathy.

Introduction

Although rare, secondary syphilis can be associated with kidney disease. In 1040 syphilitic patients proteinuria was found in 7.1% of them (Herman and Marr, 1935) and nephrotic syndrome appeared in 0.3%. Haemorrhagic nephritis has also been described (Thomas and Schur, 1946; Bhorade *et al.*, 1971). Impairment of renal function in secondary syphilis is usually transient with complete resolution either spontaneously or after penicillin treatment. Studies employing electron microscopy and immunofluorescent techniques have demonstrated immune complex deposits on the glomerular basement membrane in syphilitic nephropathy (Falls *et al.*, 1965; Braunstein *et al.*, 1970; Bhorade *et al.*, 1971; Hellier *et al.*, 1971; Kaplan *et al.*, 1972; Yuceoglu *et al.*, 1974). These, together with other studies (Gamble and Reardan, 1975; Tourville *et al.*, 1976), strongly suggest that circulating immune complexes are a pathogenetic factor in the clinical pattern of secondary syphilis. We report a case of secondary syphilis presenting as a nephrotic syndrome in which a kidney biopsy showed deposits in the glomeruli indicating an immune complex nephritis.

Investigation of serum samples from the patient revealed that circulating immune complexes were present. To our knowledge this has not previously been demonstrated in syphilitic nephropathy.

Case report

A 37-year-old woman was admitted to the Department of Medicine C, Aarhus Kommunehospital, Denmark in July 1976. The patient's symptoms had started three weeks before with arthralgia, lethargy, headache, and vomiting. Ten days before admission to hospital the patient developed generalised oedema, and five days later a papular eruption on the trunk as well as on the palms of the hands and soles of the feet. On admission examination of the heart, lungs, and abdomen was normal. Blood pressure was normal during the entire admission.

Laboratory findings revealed heavy proteinuria of about 10 g a day. Investigation of the urinary sediment showed haematuria with 10-20 erythrocytes and 2-5 leucocytes per high-powered field, and numerous hyaline casts and fat bodies. No erythrocyte casts were present. Blood urea: 49 mg/100 ml (8.1 mmol/l), serum creatinine: 1.2 mg/100 ml (106.1 µmol/l) and creatinine clearance: 60-70 ml/min. Blood electrolytes and total CO₂ were normal. Total serum protein was 4.9 g/100 ml (49 g/l), serum albumin 1.8 g/100 ml (18 g/l), serum cholesterol 286-328 mg/100 ml (7.4-8.4 mmol/l), and sedimentation rate 126 mm/h. The patient was anaemic with a haematocrit value of 27%. Leucocyte and platelet counts were normal. Alkaline phosphatase was elevated: 348 units/l (normal value below 220 units/l). Bilirubin and serum aspartate aminotransferase (SGOT) were normal. Rose-Waaler test and lupus erythematosus-factor gave negative results. No β-haemolytic streptococci or other pathogenic micro-organisms were present in

Address for reprints: J. Sølling, Department of Medicine C, Aarhus Kommunehospital, DK 8000, Denmark

Received for publication 8 August 1977

throat or urine cultures. Electrocardiogram, chest x-ray, and intravenous pyelography were normal.

The Wassermann reaction was positive: 1:12, the Kahn reaction was positive 1:8, Meinicke's reaction strongly positive, and the *Treponema pallidum* immobilisation test was also positive. A Wassermann reaction performed one year earlier had been negative. Percutaneous renal biopsy was performed one week after admission before treatment with procaine penicillin retard 600 000 units a day (PAM). The clinical course is shown in Fig. 1. The nephrotic syndrome, already in partial remission before treatment, disappeared completely. The patient left hospital after three weeks without any signs of oedema and with normal kidney function and no proteinuria.

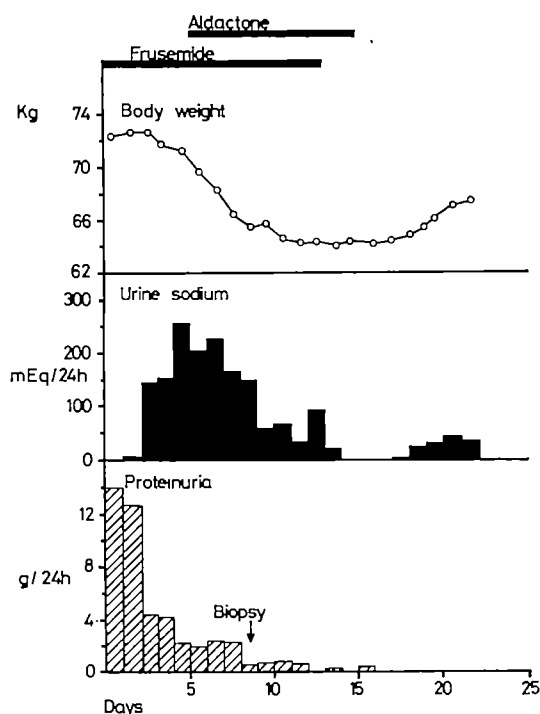


Fig. 1 The clinical course of the 37-year-old woman.

Materials and methods

Immune complexes in blood were determined by complement C1q-binding-activity (C1q-BA) (Nydegger *et al.*, 1974; Sølling *et al.*, 1978) and by an anticomplementary method modified after Mowbray (Mowbray *et al.*, 1973; Sølling *et al.*, 1978). Normal value for C1q-BA was below 15% precipitation.

The complement components C1q, C4, C3, and C3PA were determined by single radial immune diffusion according to the method of Mancini *et al.* (1965). Antibodies and protein standards for C4, C3, and C3PA from Behringwerke were used. Serum from a healthy donor was used as reference for all C1q measurements.

The percutaneous renal biopsy specimen measuring 45 mm was divided into three portions. One was fixed in Carnoy's solution and embedded in paraffin for light microscopy. Another, for electron microscopy, was fixed in 3% glutaraldehyde, postfixed in osmic tetroxide, and embedded in Vestopal W staining on the block by lead citrate and uranyl acetate. The third part was prepared for immunofluorescence, but this specimen did not contain any glomeruli.

Results

Before treatment the serum complement C1q and C4 were slightly elevated and C3 and C3PA normal. Thus no complement consumption was demonstrated. During treatment with PAM only small variations in serum complement were seen. After treatment the complement fractions declined to normal values (Fig. 2).

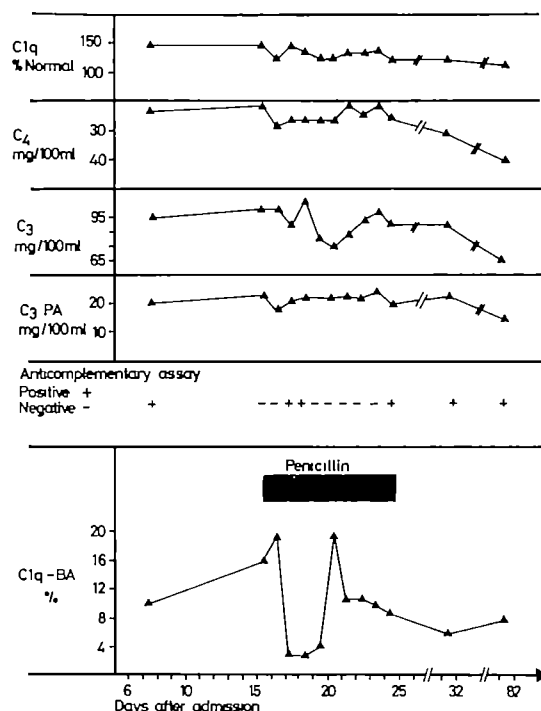


Fig. 2 The results of measurements of serum C1q, C4, C3, and C3PA complement and the results of measurements of immune complexes with the anticomplementary assay and C1q-BA.

The anticomplementary assay was positive before and the day after penicillin treatment was initiated. During treatment large fluctuations in C1q-BA were seen with normal and elevated values. After treatment the C1q-BA was normal (Fig. 2).

The biopsy specimen prepared for light microscopical examination contained 35 glomeruli. All were normal without any hypercellularity. The mean

glomerular cell count (all three cell types) was 120 per cross-section, which is within 95% confidence limits in our normal material. The capillary walls were thin and regular without spikes. There was no mesangial widening and no adhesions. Tubules, vessels, interstitium, and medulla were also normal. Total glomerular cross-sections from three glomeruli were investigated by electron microscopical exami-

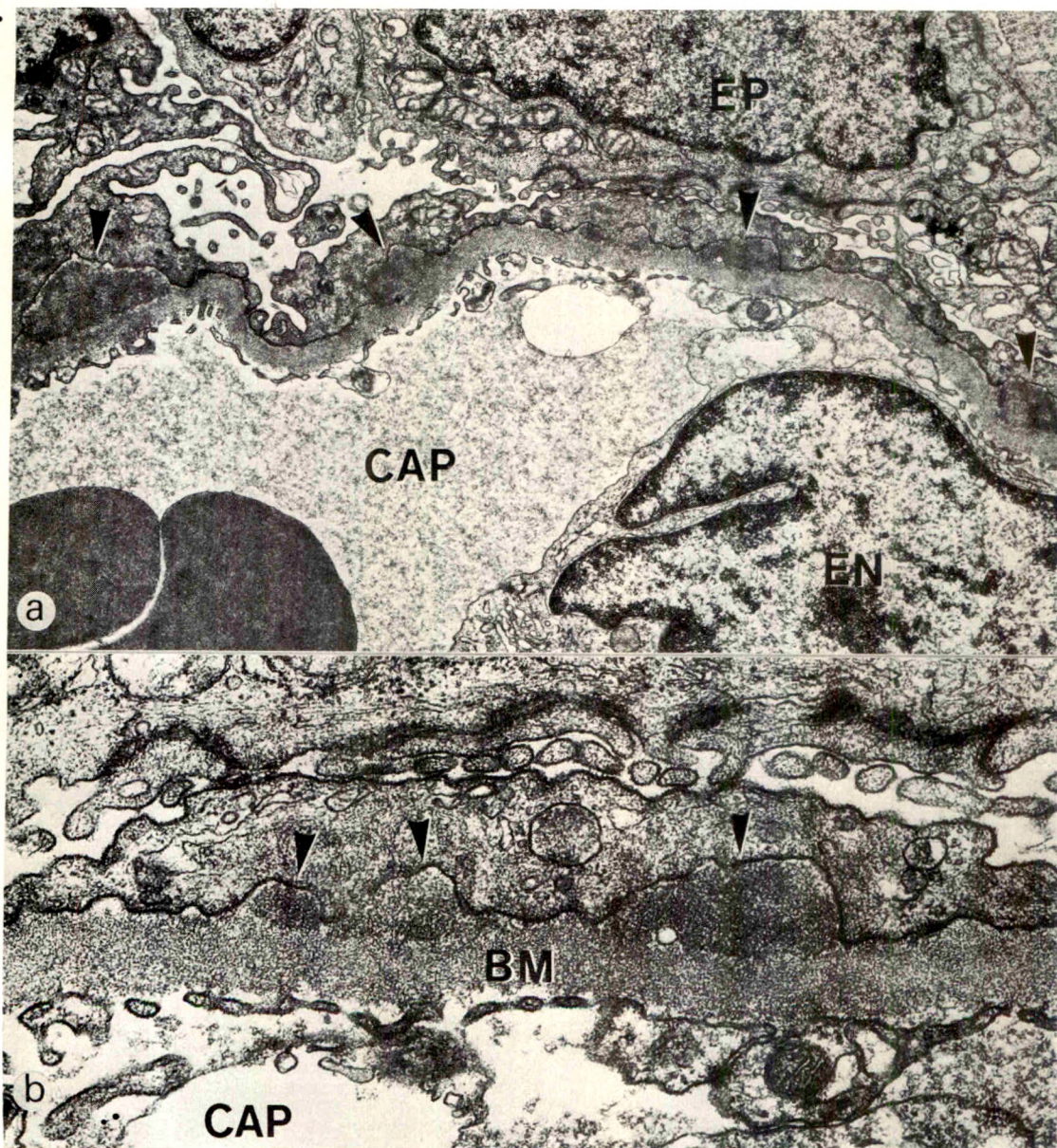


Fig. 3 Electron micrographs from glomerular capillary wall. Several accumulations of electron dense deposits (humps) are situated subepithelially (arrows) (a) $\times 12000$; (b) $\times 33600$.

EP=visceral epithelial cell; CAP=capillary lumen; EN=endothelial cell; BM=basement membrane.

nation together with several selected fields at larger magnification. In all glomeruli a multitude of hump-like subepithelial dense deposits were seen on the capillary basement membrane (Fig. 3). The epithelial foot processes were fused (retracted) and there was a severe villus-like degeneration of epithelial cells.

Discussion

The generally accepted criteria for diagnosing syphilitic nephropathy, as specified by Bhorade *et al.* (1971), were found in our patient: the clinical picture of secondary syphilis, an elevation of Wassermann reaction titre from 0 to 12 as an indication of recent infection, spontaneous remission of the nephrotic syndrome accelerated by penicillin, and no signs of other renal disease.

When Clq-binding-activity (Clq-BA) and anti-complementarity (AC) can be demonstrated, circulating immune complexes are generally accepted as being present (Mowbray *et al.*, 1973; Nydegger *et al.*, 1974). The simultaneous demonstration of Clq-BA, AC, and dense deposits in glomeruli strongly suggest that circulating immune complexes are a pathogenic factor in syphilitic nephropathy. Similar findings have been described in systemic lupus erythematosus (Nydegger *et al.*, 1974; Johnson *et al.*, 1975), but not previously in syphilis. Circulating immune complexes may also be of pathogenic importance in the liver and skin lesions of secondary syphilis (Hahn, 1943). Our patient showed a slightly elevated alkaline phosphatase remitting to normal after penicillin treatment.

Determination of Clq-BA and AC started on the eighth day after admission, 18 days after appearance of the patient's oedema. Consequently we cannot demonstrate any relationship between Clq-BA, AC, and the presence of proteinuria.

Remarkably large variations in Clq-BA and AC were observed during the period of penicillin treatment. An explanation might be that irregular lysis of the treponemes as a result of the penicillin gave rise to fluctuating levels of antigen-antibody complexes. Another possibility is that immune complexes might be present only intermittently in the circulation (Agnello *et al.*, 1976). The latter suggestion is supported by the fact that immune complexes can be detected only in a certain percentage of patients with diseases such as systemic lupus erythematosus, in which immune complexes are thought to be important.

Serum complement was normal or slightly elevated. At the start of penicillin treatment, a temporary decrease in complement was observed. Similar findings have been reported by Fulford *et al.* (1976) who observed a decrease during the first

hours after administration of penicillin, correlating with the appearance of the Jarisch-Herxheimer reaction. No Jarisch-Herxheimer reaction was observed in our patient.

In antibody elution studies performed on kidney biopsy material, Gamble *et al.* (1975) have demonstrated the presence of antitreponemal antibody within the glomerular deposits. Using an indirect fluorescent antibody technique, Tourville *et al.* (1976) demonstrated the presence of treponemal antigen in the glomerular deposits. The present study supports both these reports that syphilitic nephropathy is caused by circulating immune complexes.

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Sexually transmitted diseases

Extract from the Annual Report of the Chief Medical Officer of the Department of Health and Social Security for the year 1976

The sexually transmitted diseases continue to be controlled by the efficiency and co-ordination of all grades of staff in the clinics. Most patients attend on their own initiative but increasing numbers are referred by their general practitioners or as the result of contact tracing by health workers based at the clinics. The steady and alarming rate of increase during the 1960s has been reduced in the 1970s. For example—between 1966 and 1970 there was a 42% increase in post-pubertal gonorrhoea, whereas there was only a 4.7% rise between 1971 and 1975.

Syphilis, gonorrhoea, and chancroid

Table 1 shows the number of cases of syphilis, gonorrhoea, and chancroid reported from clinics in England in the first six months of 1976, compared with the figures for the second six months of 1975. Table 2 gives a similar comparison for cases of the other sexually transmitted diseases.

Rates of infection per 100 000 of population (Tables 3 and 4) continued to compare favourably

Table 1 *Cases of syphilis, gonorrhoea, and chancroid reported in the first six months of 1976, with figures for the second six months of 1975 in parentheses*

	Numbers of cases		
	Total	Males	Females
Syphilis			
Early	1179 (1180)	1001 (1002)	178 (178)
Primary and secondary only	819 (835)	719 (727)	100 (108)
Late	681 (613)	468 (406)	213 (207)
Congenital	62 (79)	20 (43)	42 (36)
Gonorrhoea			
All forms	26 670 (31 774)	16 871 (20 198)	9799 (11 576)
Post-pubertal	26 642 (31 747)	16 868 (20 189)	9774 (11 558)
Under 16	283 (294)	46 (62)	237 (232)
16-19	5072 (6395)	2035 (2593)	3037 (3802)
20-24	8796 (10 525)	5335 (6560)	3461 (3965)
25-34	9138	6760	2378
35-44	2561 (14 533)	2024 (10 974)	537 (3559)
45 and over	792	668	124
Chancroid	26 (42)	22 (40)	4 (2)

Figures for the second half of 1976 will be published in next year's Report.

Table 2 *Other sexually transmitted diseases reported in the first six months of 1976, with figures for the second six months of 1975 in parentheses*

	Numbers of cases		
	Total	Males	Females
Lymphogranuloma venereum	14 (19)	13 (18)	1 (1)
Granuloma inguinale	10 (6)	7 (5)	3 (1)
Non-specific genital infection (NSGI)	43 285 (44 401)	34 379 (35 784)	8906 (8617)
NSGI with arthritis	241 (250)	228 (240)	13 (10)
Trichomoniasis	9352 (10 337)	767 (773)	8585 (9564)
Candidosis	17 842 (18 374)	3230 (3027)	14 612 (15 347)
Scabies	1212 (1438)	988 (1 140)	224 (298)
Pediculosis pubis	2544 (2657)	1795 (1839)	749 (818)
Genital herpes	3341 (3319)	2149 (2170)	1192 (1149)
Genital warts	10 918 (10 028)	7160 (6476)	3758 (3552)
Genital molluscum	455 (373)	327 (245)	128 (128)
Other treponemal diseases	540 (506)	349 (315)	191 (191)
Other conditions requiring treatment in a centre	18 846 (19 820)	12 189 (13 205)	6657 (6615)
*Other conditions not requiring treatment in a centre	43 860 (43 066)	27 457 (26 775)	16 403 (16 291)

Figures for the second half of 1976 will be published in next year's Report.

*Other conditions referred elsewhere are included in these totals.

Table 3 The venereal diseases—new cases per 100 000 population, by age, seen at hospital clinics in England 1972–76

Disease	1972			1973			1974			1975			First half-year 1976		
	Male	Female	Total	Male	Female	Total	Male	Female	Total	Male	Female	Total	Male	Female	Total
Early syphilis															
All ages	6.00	1.25	3.56	7.98	0.90	4.59	8.43	1.56	4.91	8.52	1.41	4.87	4.43	0.75	2.54
Early syphilis (primary and secondary only)															
All ages	4.46	0.77	2.56	5.75	0.90	3.26	6.17	1.06	3.55	6.21	0.85	3.46	3.18	0.42	1.76
Under 16	0.02	0.12	0.07	0.07	0.07	0.07	0.05	0.04	0.04	0.05	0.05	0.03	0.13	0.23	0.18
16 and 17	3.84	1.45	2.67	3.02	2.06	2.56	2.63	3.23	2.92	4.71	1.50	3.14	4.30	1.50	2.93
18 and 19	7.47	4.32	5.93	9.60	4.24	6.99	11.50	5.37	8.27	8.89	4.37	6.92	10.23	1.79	6.11
20–24	13.09	3.83	8.49	15.52	4.51	10.07	18.74	4.59	11.74	17.86	3.83	10.97	16.25	0.25	1.49
25 and over	5.24	0.49	2.73	7.00	0.65	3.64	7.27	0.81	3.86	7.40	0.68	3.84	2.07	0.89	1.47
Late syphilis															
All ages	3.40	1.66	2.50	3.52	1.58	2.52	3.64	1.59	2.58	3.54	1.67	2.58	0.09	0.18	0.13
Congenital syphilis															
All ages	0.29	0.40	0.34	0.27	0.43	0.35	0.25	0.45	0.35	0.34	0.38	0.36	0.09	0.18	0.13
Gonorrhoea (post-pubertal)															
All ages	155.64	77.10	115.28	167.40	87.04	126.10	165.19	87.00	125.06	165.20	90.56	126.91	74.61	41.05	57.40
Under 16	1.81	7.36	4.52	2.53	8.09	5.24	1.99	8.19	5.01	1.83	8.02	4.84	1.98	10.73	12.15
16 and 17	144.61	362.92	251.24	167.40	405.56	283.59	165.94	376.75	268.74	158.00	393.11	272.81	145.72	227.66	185.75
18 and 19	487.50	575.43	530.40	551.55	664.28	606.44	546.94	665.91	603.04	548.21	660.44	616.92	326.70	221.72	275.39
20–24	675.71	393.06	535.40	751.15	450.75	602.41	759.88	464.05	613.63	748.91	482.94	618.25	54.79	16.25	34.74
25 and over	143.15	36.26	86.61	150.80	41.61	93.08	147.19	41.52	91.27	147.83	42.53	92.24	0.10	0.02	0.06
Chancroid															
All ages	0.21	0.01	0.11	0.15	0.01	0.08	0.17	0.02	0.09	0.29	0.03	0.15	0.03	0.02	0.06

*These rates were based on fewer than 10 events and consequently their reliability as a measure may be affected.
†Indicates that there were no events.

Table 4 Other sexually transmitted diseases and other conditions—new cases per 100 000 population at all ages and by sex seen at hospital clinics in England 1971–76

Disease	1972			1973			1974			1975			First half-year 1976		
	Male	Female	Total	Male	Female	Total	Male	Female	Total	Male	Female	Total	Male	Female	Total
Lymphogranuloma venereum	0.24	0.02	0.13	0.23	0.03	0.13	0.17	0.03	0.10	0.15	0.02	0.08	0.06	0.03	0.03
Granuloma inguinale	0.01	0.01	0.01	0.02	0.01	0.02	0.03	0.02	0.02	0.04	0.02	0.03	0.03	0.01	0.02
Non-specific genital infection	277.65	60.61	166.13	301.70	62.54	178.80	306.64	62.54	181.35	306.59	69.58	185.01	152.05	37.41	93.25
Non-specific genital infection with arthritis	1.99	0.10	1.02	1.93	0.08	0.98	1.73	0.09	0.89	2.04	0.13	1.06	1.01	0.05	0.52
Trichomoniasis	6.82	73.38	41.02	7.16	73.48	41.22	6.28	73.81	40.94	6.62	77.03	42.74	3.39	36.06	20.15
Candidosis	19.34	107.16	64.46	21.66	113.20	68.68	23.17	114.21	72.05	25.26	123.18	75.49	14.29	61.37	38.44
Scabies	9.93	2.25	5.99	9.36	1.90	5.53	9.91	2.11	5.90	9.97	2.27	6.02	4.37	0.94	2.61
Pubic lice (pediculosis pubis)	13.45	4.50	8.85	13.97	4.73	9.23	15.85	5.68	10.63	16.01	6.25	11.01	7.94	3.15	5.48
Herpes simplex	13.84	5.31	9.46	14.71	6.48	10.48	15.36	7.25	11.30	18.29	8.72	13.38	9.50	5.01	7.20
Warts (condylomata acuminata)	45.54	23.42	34.17	51.19	26.48	38.50	55.09	26.36	40.34	57.86	29.62	43.38	31.67	15.78	23.52
Molluscum contagiosum	2.03	0.74	1.37	2.13	0.74	1.42	2.04	0.86	1.43	2.31	0.97	1.62	1.45	0.54	0.98
Other treponemal diseases	2.55	1.17	1.84	2.43	1.32	1.86	3.13	1.50	2.30	2.74	1.61	2.16	1.54	0.80	1.16
Other conditions requiring treatment in a centre	101.85	39.32	69.72	111.10	44.0	76.67	109.42	45.90	76.82	112.97	53.66	82.54	53.91	27.96	40.60
Other conditions not requiring treatment in a centre	208.90	110.19	158.18	235.70	126.30	179.50	231.93	130.59	179.92	233.85	134.89	183.09	119.04	67.57	92.64

*These rates were based on fewer than 10 events and consequently their reliability as a measure may be affected.

with those of other developed countries in the world, where reliable statistics are available.

Since the Council of Europe recommendations came into force in 1976 the age bands for the collection of statistics on sexually transmitted diseases have been changed. Because of this change direct comparisons in certain groups (see bottom of Table 1) are not possible, and the bracketing of certain groups has been necessary. In addition there have been changes in the classification of complications of gonorrhoea, these being modifications made, in conjunction with the Scottish Home and Health Department, of the Council of Europe recommendations.

It is possible that some of these new categories were not reported by clinics in the first half of 1976 and more reliable data will be available next year.

Imported cases

In the first half of 1976, 113 out of 819 cases (13.8%) of early infectious syphilis (primary and secondary) were believed to have been contracted abroad (126 out of 835 (15.1%) in the second half of 1975). The corresponding figures for post-pubertal gonorrhoea were 863 out of 26 642 (3.2%) (957 out of 31 747 (3%) in the second half of 1975).

Contacts

In the first half of 1976, 1270 contacts of cases of syphilis were approached to attend a clinic. As a result, 548 males and 223 females attended; of these, 153 males and 90 females were found to have syphilis. The corresponding figures for gonorrhoea were: 20 827 contacts were approached to attend a clinic; 5048 males and 7661 females attended and of these 2740 males and 5684 females were found to have gonorrhoea.

The present position

Hospital medical staff engaged in genitourinary medicine in England and Wales at 30 September 1976 totalled 210 (185.1 whole-time equivalents (WTE), including 103* (94.7 WTE) consultants, 32 (30.0 WTE) senior registrars, and 35 (31.5 WTE) registrars, compared with a total of 206 (180.8 WTE) at 30 September 1975, with 100 (91.1 WTE) consultants, 29 (27.9 WTE) senior registrars, and 37 (33.5 WTE) registrars. At 30 September 1975, 76 out of 110 consultants specialising in genitourinary medicine in Great Britain were full time.

In 1976 the proceedings of the Anglo-American conference on sexually transmitted diseases, held in London in June 1975, were published (Catterall and Nicol, 1976). The conference was sponsored by the Royal Society of Medicine and was introduced

by the President of the Royal Society of Medicine, Sir John Stallworthy, and the Chief Medical Officer of the DHSS, Sir Henry Yellowlees. Papers were presented by British and American speakers on: (1) World trends in STD, (2) Control through health care systems, (3) The changing clinical picture in gonorrhoea and non-gonococcal infections, (4) Immunology of *Chlamydia*, (5) Virus diseases: (i) Genital herpes, (ii) Hepatitis, (6) Syphilis, (7) Responsibility for education and prevention programmes, and (8) Reflections on the conference.

A summary report is available (World Health STD was organised in Vienna by WHO Regional Office for Europe in 1976. Topics for the meeting were:

1. Extent and trends of sexually transmitted disease
2. Case finding (a) by screening (b) by contact tracing
3. Economic aspects
4. Health education
5. Training of personnel

A summary report is available (World Health Organisation, 1976).

Research projects in sexually transmitted disease continue to be supported by the MRC and the DHSS. In addition, DHSS provided special financial support which enabled 10 doctors, nurses, and technicians to travel abroad for research purposes during 1976. Their reports again show the value of these visits.

Towards the end of 1976 a new strain of gonococcus resistant to penicillin, because it produced penicillinase (β lactamase), was reported in the United States and in this country, with contact sources in South East Asia and Africa. Action has been taken by WHO to monitor the emergence of this strain which although completely resistant to penicillin is at present sensitive to spectinomycin (Lancet, 1976; Public Health Laboratory Service, 1976; Wilkinson *et al.*, 1976). The DHSS took the necessary steps to ensure that physicians in charge of clinics were aware of this development.

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*In the Report the figure of 193 was erroneously given

Abstracts

These selected abstracts and titles from the world literature are arranged in the following sections:

Syphilis and other treponematoses

(Clinical and therapy; serology and biological false positive phenomenon; pathology and experimental)

Gonorrhoea

(Clinical; microbiology; therapy)

Non-specific genital infection

Reiter's disease

Trichomoniasis

Candidosis

Genital herpes

Other sexually transmitted diseases

Public health and social aspects

Miscellaneous

Syphilis and other treponematoses (Clinical and therapy)

Secondary syphilis presenting as a pruritic dermatosis

G. W. COLE, R. B. AMON, AND P. S. RUSSELL (1977).

Archives of Dermatology, 113, 489-490

Neurosyphilis (Review Article)

R. D. CATTERALL (1977).

British Journal of Hospital Medicine, 17, 585-604

Syphilis (Serology and biological false positive phenomenon)

Comparison between the automated reagin test and reagin screen test methods of VDRL screening tests for syphilis in use in a routine laboratory

R. D. SIMON AND A. M. PEACOCK (1977).

Journal of Clinical Pathology, 30, 626-630

This paper compares the results of the automated reagin test (ART) with those of the reagin screen test (RST) on 1154 sera from patients attending a venereal disease clinic. TPHA tests were also performed on all sera by a modified method, which is not described, and FTA-ABS tests carried out on reactive sera.

1028 sera were negative in all three screening tests, 66 were positive in all tests, 42 gave positive TPHA and FTA-ABS tests but the ART and RST results were negative. Sixteen sera gave positive

RST but negative ART results, TPHA and FTA-ABS tests were positive on all of these. Quantitative tests on 15 sera showed that the RST gave titres two to four times greater than the ART. The RST gave clearer readings than the ART with weakly reactive sera; it is suggested that the RST in combination with the TPHA test provides a satisfactory combination for screening purposes.

A. E. Wilkinson

[Reprinted from *Abstracts on Hygiene*, by permission of the Editor.]

Test di emoagglutinazione treponemica (TPHA). Validità del micrometodo esoguito con le diluizioni dei sieri 1/20-1/40. (Treponemal haemagglutination test (TPHA). Validity of the micromethod with serum dilutions of 1/20-1/40)

E. ALESSI AND L. SCIOCCATI (1977).

Bollettino dell'Istituto Sieroterapico Milanese, 56, 102-107

The authors found that the sensitivity of the microTPHA test with sera diluted 1 in 80 was less than that of the macrotest at the same dilution, particularly in early syphilis. Microtests at dilutions from 1 in 20 to 1 in 160 were carried out in parallel with macrotests on 773 sera. Nine sera gave invalid results in the microtest owing to agglutination of the control cells, all the macrotests gave valid results. All 235 sera examined from patients thought not to have syphilis, gave negative TPI and FTA-ABS tests; three of these were positive by the micro-method and one by the macrotest. A

further 50 sera had given false positive results with standard tests as judged by negative TPI and FTA-ABS results; four of these gave positive micro- and two positive macrotests. In tests on 180 sera from patients with untreated early syphilis and 299 with treated early infections the sensitivity of the microtest at a dilution of 1 in 20 was virtually equal to that of the macrotest at 1 in 80. These results suggest that the use of a test dilution of 1 in 20 in the micromethod increases its sensitivity to that of the macromethod without undue loss of specificity; further studies are desirable to confirm this.

A. E. Wilkinson

[Reprinted from *Abstracts on Hygiene*, by permission of the Editor.]

Serologic study of specimens with borderline FTA-ABS test reactivity

D. BAERTSCHY, R. GOLUBJATNIKOV, M. STEADMAN, AND S. L. INHORN (1977).
Health Laboratory Science, 14, 177-182

Borderline FTA-ABS results were found on 2% of 23 807 sera examined at the Wisconsin State Laboratory of Hygiene and were confirmed on a second specimen of serum. Of the 479 patients concerned, 60% were women, but this probably reflects only the sex ratio of the whole group. Borderline reactivity was found in all age groups but was commoner in those 50 years of age or older. Tests for antinuclear antibody were performed on 461 of the sera; seven gave positive and eight inconclusive results. These were not limited to any age group and the presence of antinuclear antibody did not seem to

- be a common cause of borderline FTA-ABS reactions. TPHA tests were found reactive at a titre of 1 in 80 or above in only 79 of the 479 sera; positive results were commoner in patients over 50 years of age. Of the 466 patients followed-up, 11.4% were considered to have present or past syphilis, 38% were thought to be free from infection, and the records were inadequate to assess the state of the remainder.

A. E. Wilkinson

[Reprinted from *Abstracts on Hygiene*, by permission of the Editor.]

Spinal syphilis: the problem of fluorescent treponemal antibody in the cerebrospinal fluid

J. F. JOHN AND A. C. CUETTER (1977). *Southern Medical Journal*, 70, 309-311

Three cases of late onset, non-tabetic, spinal syphilis are described, in which other causes of the spinal syndromes are excluded.

Case 1 A 49-year-old woman with a sudden onset of flaccid paraplegia consistent with thrombosis of the anterior spinal artery, had been known to have positive syphilitic serology for four years and had received penicillin treatment. Her serum FTA-ABS and RPR results were positive.

The cerebrospinal fluid was acellular, with a raised protein of 42 mg/100 ml (0.42 g/l). The cerebrospinal fluid VDRL and FTA results were positive.

Case 2 A 43-year-old man had, at the age of 12 years, received treatment for syphilis and serology had remained positive for at least 14 years before he developed progressive combined disease of his posterior and lateral columns which was treated with penicillin. It worsened one year later and at that time there were 4 cells/mm³ in his cerebrospinal fluid, the protein was 49 mg/100 ml (0.49 g/l); the cerebrospinal fluid VDRL result was negative and the FTA test positive.

Case 3 A 63-year-old woman with no previous history of syphilis developed a transverse myelitis. Her serum RPR and FTA-ABS results were positive, the cerebrospinal fluid acellular with a total protein of 29 mg/100 ml (0.29 g/l) and slight elevation of the gammaglobulin fraction, the cerebrospinal fluid VDRL was negative and the FTA positive.

It is noted from these cerebrospinal fluid findings that the diagnosis of syphilis should be accepted with reluctance, but the nature of the syndromes and the positive cerebrospinal fluid FTA tests support the belief that neurosyphilis caused the myelopathic process. It is argued that failure to improve after treatment with penicillin does not exclude the diagnosis of neurosyphilis and that the cerebrospinal fluid FTA-ABS is not a reflection of the serum activity, but a separate response arising within the cerebrospinal fluid.

Michael Kelsey

Routine tests for syphilis on cerebrospinal fluid (Leading Article) (1977). *Lancet*, 2, 595

Syphilis (Pathology and experimental)

Specific direct fluorescent antibody detection of *Treponema pallidum*

K. C. DANIELS AND H. S. FERNEYHOUGH (1977). *Health Laboratory Science*, 14, 164-171

Smears of exudate from 350 lesions were sent to a laboratory for direct fluorescent antibody tests (DFT) for *Treponema pallidum* and the results compared with those of ordinary darkground (DG) examinations. 118 of the patients had syphilis; the DFT was positive in 103 of these and the DG in 87. The DFT was negative on material from all of the 232 patients who were thought not to have syphilis; the DG was positive on six of these, but the results were thought to be falsely positive. In a further 75 cases the DFT was compared with FTA-ABS tests on blood collected at the same time as the exudate. The DFT was positive in 12 and the FTA-ABS test in 14. The DFT is thought to be as reliable as the conventional DG in the diagnosis of early syphilis. It offers advantages when the lesion is on a site which may harbour commensal treponemes, such as the mouth or rectum, or when the DG is inconclusive or is not available. The need for care in preparing smears for the DFT is rightly stressed.

A. E. Wilkinson

[Reprinted from *Abstracts on Hygiene*, by permission of the Editor.]

Catabolism of glucose and fatty acids by virulent *Treponema pallidum*

N. L. SCHILLER AND C. D. COX (1977). *Infection and Immunity*, 16, 60-80

We describe a procedure which permits essentially full recovery of physiologically active *Treponema pallidum* from rabbit testicular extracts and greatly reduces contaminating tissue material. Such preparations were employed for investigations of the ability of *T. pallidum* to catabolise glucose and fatty acids. Radiospirometric studies revealed that glucose and pyruvate, but not oleate or palmitate, could be degraded to CO₂. The use of differentially labelled glucose, in conjunction with enzymatic analyses, indicated that glucose was catabolised by a combination of the Embden-Meyerhoff-Parnas and hexose monophosphate pathways. Pyruvate was degraded to CO₂ from only the carbonyl position, suggesting the absence of a functioning tricarboxylic cycle; this was substantiated by additional enzyme analyses and radiorespirometric experiments. Oleate and palmitate were incorporated but not catabolised by β -oxidation. Glucose, although catabolised, was not incorporated. The potential significance of these findings is discussed.

Author's summary

The effect of *Treponema pallidum* on mouse survival

D. J. M. WRIGHT AND F. P. WHARTON (1977). *Journal of Medical Microbiology*, 10, 245-248

Gonorrhoea (Clinical)

Acute adult respiratory distress syndrome associated with gonococcal septicaemia

J. D. MARKHAM, J. R. VILSECK, AND W. J. O'DONOHUE (1976). *Chest*, 70, 667-669

Sepsis appears to be an important factor in the causation of the acute respiratory distress syndrome in adults. A case of this syndrome caused by gonococcal septicaemia is reported in this paper.

A 40-year-old woman was given aspirin by her physician for pain in her left knee and left arm. Her symptoms increased and she reported two days later at the local hospital. By this time she was acutely ill. She was transferred to the intensive care unit after 40 ml of cloudy

yellowish fluid had been aspirated from her left knee.

The patient was uncooperative, delirious, and breathing rapidly. Her blood pressure was 116/64 mmHg, pulse 102/min, and respiration 22–28/min. The white cell count was 18 800/ μ l (18.8×10^9 /l), predominantly polymorphonuclears. Examination showed that the lungs and heart were normal, apart from a marked tachycardia. There was an effusion in the left knee, which was swollen and tender. Pelvic examination showed a profuse yellowish-green purulent vaginal discharge.

The patient was sedated with diazepam, and treatment with digoxin was begun. Antibiotic therapy was continued with intravenous ampicillin, but this was changed to intravenous penicillin G 10 megaunits daily and probenecid 1 g daily, when the cervical culture was found to be positive for *N. gonorrhoeae*. The blood and joint fluid cultures were negative. After arterial blood gas analysis, intensive respiratory care was begun with methylprednisone intravenously and frusemide intramuscularly; acute respiratory distress syndrome was suspected, and serial radiograms confirmed this later. The patient made a rapid recovery and was discharged from hospital fully ambulatory 13 days after admission.

The authors point out that the increasing incidence of gonorrhoea will inevitably be associated by a greater incidence of complicated and unusual cases presenting to physicians.

C. S. Ratnatunga

Gonococcal meningitis and ventriculitis in the presence of a ventriculoperitoneal shunt

R. C. NOBLE AND R. M. COOPER (1977).
Sexually Transmitted Diseases, 4, 9–11

Gonorrhoea (Microbiology)

Survival of *Neisseria gonorrhoeae* on surfaces

T. ELMROS (1977).
Acta Dermato-venereologica, 57, 177–180

The aim of the study was to investigate the survival of *N. gonorrhoeae* outside the body. When pus from cases of urethral gonorrhoea was kept on a glass surface and incubated at 22°C, at the end of

24 hours gonococci could be cultured in three out of four cases. After 48 hours' incubation at this temperature, no organisms could be isolated. The organisms appeared to survive in pus much better at a lower temperature, isolates being obtained from four of 10 specimens kept at a temperature of 4°C for two days. Similar results were obtained using a linen surface.

When organisms were incubated with serum at 22°C, it was shown that a slow decrease in the number of viable gonococci occurred during the first 60 minutes. In the succeeding 30 minutes, damage rapidly developed so that by 90 minutes only 0.1% of the number of organisms in the initial inoculum were recoverable. There then followed a gradual decline in the number of viable organisms, and by 24 hours only 0.002% of the inoculum survived. When the organisms were suspended in saline in the place of serum, the same initial response was obtained, but there was no gradual decline after 90 minutes, all organisms having been affected by this time.

It was suggested that dehydration was the main external cause of organismal death and that the protective effect of serum and secretions was attributable to the hygroscopic properties of these fluids, diminishing water loss. An alternative hypothesis was that proteins in these fluids absorbed factors toxic to the bacteria. That the survivors were not mutants resistant to desiccation was shown by culturing the surviving organisms at 24 hours, and showing that the survival curves were identical with those previously obtained with the less hardy organisms.

A. McMillan

Growth pattern and cell division in *Neisseria gonorrhoeae*

B. WESTLING-HÄGGSTRÖM, T. ELMROS, S. NORMARK, AND B. WINBLAD (1977).
Journal of Bacteriology, 29, 333–342

The Gram-negative coccus *Neisseria gonorrhoeae* was found to grow regularly in at least two dimensions. Growth proceeded at a linear rate sequentially in each dimension. Growth in the second dimension (former width) was initiated slightly before the pole-division plane distance equalled the cell width. Penicillin treatment localised presumptive growth zones to the existing septum region. It

was suggested that new growth zones were always formed perpendicular to the longitudinal axis created in the incipient daughter cells of a dividing coccus. Neither penicillin nor nalidixic acid induced filaments of *N. gonorrhoeae*. Such structures could nevertheless be formed in the rod-shaped species *Neisseria elongata*. *N. gonorrhoeae* divides by septation; however, complete septal structures with separated cytoplasm were uncommon. It is proposed that *N. gonorrhoeae* be regarded as a short rod which always extends parallel to the actual longitudinal axis and which never undergoes a rod-sphere transition.

Author's summary

Isolation and characterisation of a rough colony type of *Neisseria gonorrhoeae*

N. F. JACOBS, S. J. KRAUS, C. THORNSBERRY, AND J. BULLARD (1977).
Journal of Clinical Microbiology, 5, 365–374

This new colony type was found in eight of 180 primary cultures from men with gonococcal urethritis. BBL GC agar base supplemented with IsoVitalX (10 ml/l) was used for isolation and strains were identified by fermentation tests. After 20 hours' incubation at 35°C in candle jars the rough colonies were 0.3–0.6 mm in diameter with a highly granular surface and irregular periphery. In six of the primary cultures they predominated with only scattered type 1 and type 2 colonies; in two cases 90% of the colonies were type 1 and 10% the rough form. The rough characteristic could be maintained by selective subculture, but if transfers were made non-selectively, smooth type 3 and 4 colonies became predominant after about six passages. The rough forms were pilated and autoagglutinated in saline. Sensitivity to ampicillin, penicillin, tetracycline, and spectinomycin was not significantly different for rough colonies and type 1 colonies derived from the same primary isolates. The rough forms produced infection in subcutaneous chambers in guinea-pigs but were gradually supplanted by type 1 colonies. After inoculation of chambers with type 1 colonies from strains showing rough variants the predominant colony type was always type 1 with only occasional rough colonies.

A. E. Wilkinson

Simplified complete medium for the growth of *Neisseria gonorrhoeae*

R. T. JONES AND R. S. TALLEY (1977).
Journal of Clinical Microbiology, 5, 9-14

An enriched medium for growth of *Neisseria gonorrhoeae* was tested using 10 laboratory and 67 (in methods 37) clinical isolates. The basal medium (in g/l) comprised polypeptone peptone 15, K_2HPO_4 4, KH_2PO_4 1, NaCl 5, soluble starch 1, and purified agar (Difco) 10. The supplements in μ g/ml were glucose 5000, thiamine 2, ferric nitrate 5, cysteine 5, hypoxanthine 5, and uracil 10.

Comparative studies using GC agar base (BBL) and IsoVitaleX 10 ml/l showed no increased plating efficiency for the new medium, and incorporation of VCN inhibitor (BBL) 10 ml/l did not affect isolation. Culture on liquid media made as above, but with sodium bicarbonate 500 μ g/l substituted for starch and agar, yielded a generation time of 30.5 minutes in the log growth phase for *N. gonorrhoeae* with an exponential decline in viable bacteria (colony forming units) from 8 hours where the count was 5×10^9 cfu/ml to 10^4 cfu/ml at 48 hours. Neutralisation of toxicity of different sources of agar by starch was also studied.

D. J. M. Wright

In vitro inhibition of growth of *Neisseria gonorrhoeae* by genital micro-organisms

D. KAYE AND M. E. LEVISON (1977).
Sexually Transmitted Diseases, 4, 1-3

The ability of micro-organisms present in titres of over 10^5 /ml in the vaginal or cervical secretions to inhibit growth of *N. gonorrhoeae* in vitro was tested. Study of a strain of *N. gonorrhoeae* against 77 micro-organisms demonstrated that most strains of *S. epidermidis*, *S. aureus*, and the one *Gaffkya anaerobia* interfered with the growth of the *N. gonorrhoeae*. A few strains of *S. viridans*, *Neisseria*, *Candida*, and *Bifidobacterium* demonstrated interference. No strains of enterococcus, diptheroids, aerobic *Lactobacillus*, *Peptostreptococcus*, *Peptococcus*, anaerobic *Lactobacillus*, or *Veillonella* demonstrated interference.

Authors' summary

Binding of progesterone to *Neisseria gonorrhoeae* and other Gram-negative bacteria

R. D. MILLER AND S. A. MORSE (1977).
Infection and Immunity, 16, 115-128

Interaction of *Neisseria gonorrhoeae* with guinea-pig defence mechanisms in subcutaneously implanted chambers

D. R. VEALE, D. SEN, C. PENN, H. FINCH, H. SMITH, AND K. WITT (1977).
FEMS Microbiology Letters, 1, 3-6

Evaluation of a serologic test for gonorrhea in a low-risk female population

M. NELSON, E. J. PORTONI, M. ISHIDA, AND M. J. FELDMAN (1977).
Southern Medical Journal, 70, 316-319

Gonorrhoea: Therapy

The bactericidal action of spectinomycin on *Neisseria gonorrhoeae*

M. E. WARD (1977).
Journal of Antimicrobial Chemotherapy, 3, 323-329

The *in vitro* bactericidal activity for gonococci of four different antibiotics—penicillin, tetracycline, kanamycin, and spectinomycin—at their appropriate peak serum concentrations was compared. Spectinomycin was much more rapidly bactericidal for gonococci than the others; this contrasts sharply with the bactericidal action of spectinomycin for *Escherichia coli* at concentrations as high as 1 mg/ml. This rapid bactericidal action may be advantageous for any attempts to eradicate penicillinase-producing gonococci. Electron microscope studies showed that spectinomycin produces alterations in gonococcal surface morphology leading to lysis. No significant differences were detected by sodium dodecyl sulphate polyacrylamide gel electrophoresis between the outer envelope proteins of gonococci grown in the presence or absence of spectinomycin, but there was evidence of ultrastructural damage to the cytoplasmic membrane. It is suggested that spectinomycin may inhibit the synthesis of a critical cytoplasmic membrane protein leading to ultimate impairment of the osmotic integrity of the cell.

Author's summary

Pivampicillin with probenecid in the treatment of gonorrhoea

M. A. WAUGH AND K. C. NAYYAR (1977).
Clinical Trials Journal, 14, 152-155

Penicillinase-producing *Neisseria gonorrhoeae* (Letter)

R. M. ROBINS-BROWNE, M. C. GAILLARD, H. J. KOORNHOF, AND A. C. MAUFF (1977).
South African Medical Journal, 51, 568

The sensitivity of gonococci to penicillin

A. E. WILKINSON (1977).
Journal of Antimicrobial Chemotherapy, 3, 197-198

Control of penicillinase-producing gonococci (Leading Article) (1977).

British Medical Journal, 1, 1617-1618

Non-specific genital infection

The etiology of non-gonococcal urethritis in men attending a venereal disease clinic

J. L. WONG, P. A. HINES, M. D. BRASHER, G. T. ROGERS, R. F. SMITH, AND J. SCHACHTER (1977).
Sexually Transmitted Diseases, 4, 4-8

Non-gonococcal urethritis was identified as a main reason for men attending our venereal disease clinic. The prevalence of several agents that might cause non-gonococcal urethritis was determined. Attempts were made to isolate gonococci, chlamydiae, *Ureaplasma urealyticum*, trichomonads, *Candida* sp. and *Corynebacterium vaginale* from urethral swabs from 307 men. Chlamydiae were recovered from 31% of the 67 men with non-gonococcal urethritis compared with only 4% of 86 asymptomatic men without pyuria. Unexpectedly, cultures from only 4% of the 99 men with gonorrhoea also yielded chlamydiae. *Ureaplasma urealyticum* was recovered from nine of 27 asymptomatic men (33%), 16 of 30 men with non-gonococcal urethritis (53%), and 16 of 38 men with gonorrhoea (42%). These differences were not statistically significant. However, when *Chlamydia*-positive men were excluded from the analysis, a significant association was found for *U. urealyticum* and non-gonococcal urethritis. Twelve of 18 (76%) men with non-chlamydial non-gonococcal urethritis yielded the organism compared with eight of 26 (31%) men without urethritis. The other organisms sought were not often recovered and could not be associated with non-gonococcal urethritis.

Authors' summary

Etiology of non-gonococcal urethritis. Evidence for *Chlamydia trachomatis* and *Ureaplasma urealyticum*

W. R. BOWIE, S. P. WANG,
E. R. ALEXANDER, P. S. FORSYTH,
H. M. POLLOCK, J. S. L. LIN,
T. M. BUCHANAN, AND K. K. HOLMES
(1977). *Journal of Clinical Investigation*,
59, 735-742

Chlamydia trachomatis, *Ureaplasma urealyticum* (T. mycoplasma), and *Haemophilus vaginalis* have previously been considered possible aetiological agents in non-gonococcal urethritis (NGU). In this study, current *C. trachomatis* infection was confirmed by culture and/or micro-immunofluorescence serology in 26 of 69 men experiencing a first episode of NGU, and in one of 39 without urethritis. Serum IgM immunofluorescent antibody to *Chlamydia* was demonstrated in 16 of 20 men with *Chlamydia* culture positive NGU, and in three of 39 with *Chlamydia* culture negative NGU, and in none of 34 without urethritis. Nine of 10 culture positive men with symptoms of ≤ 10 days developed immunofluorescent antibody seroconversion in paired sera. *U. urealyticum* was isolated significantly more often and in significantly higher concentrations from first voided urine from *Chlamydia*-negative cases of NGU than from *Chlamydia*-positive NGU. Ureaplasma-specific antibody titres increased fourfold in six men, four of whom had negative cultures for ureaplasma. *H. vaginalis* was isolated from 19 of 33 men with no urethritis and from two of 69 with NGU. *C. trachomatis* is susceptible and *U. urealyticum* is resistant to sulphonamides. A 10-day course of sulphafurazole (Sulphisoxazole) therapy produced improvement in 13 of 13 *Chlamydia*-positive, ureaplasma-negative, and in only 14 of 29 *Chlamydia*-negative, ureaplasma-positive NGU cases ($P < 0.002$). Thus, culture, serology, and response to therapy support the aetiological role of *Chlamydia* in NGU. Quantitative culture and response to therapy suggest *U. urealyticum* may cause many cases of *Chlamydia*-negative NGU.

Authors' summary

The developmental cycle of *Chlamydia trachomatis* in McCoy cells treated with cytochalasin B

P. STIRLING AND S. RICHMOND (1977).
Journal of General Microbiology, 100,
31-42

The growth of a genital trachoma-inclusion conjunctivitis agent strain of *Chlamydia trachomatis* in McCoy cells treated with cytochalasin B was studied by quantitative infectivity estimations and by light and electron microscopy. Provided that infection of the monolayer was initiated by centrifuging the infectious particles on to the cells before incubation, this chlamydial strain grew as fast and to as high a titre (approximately 10^7 inclusion-forming units (ifu) per culture) as those chlamydiae which infect cell cultures *in vitro* without centrifugation. Each ifu inoculated yielded approximately 600 ifu, and extracellular infectivity was detected soon after intracellular infectivity appeared. Inclusions were recognised by fluorescent antibody staining techniques early in the developmental cycle when cultures were not infectious and when only reticulate bodies were seen by electron microscopy. Inclusions were recognised in Giemsa-stained preparations examined by darkground microscopy only when elementary bodies appeared in the inclusions. Iodine staining was not a reliable indicator of either the number of inclusions present or of their infectivity.

Authors' summary

Antimicrobial activity of several antibiotics and a sulfonamide against *Chlamydia trachomatis* organisms in cell culture

C.-C. KUO, S.-P. WANG, AND T. GRAYSTON
(1977). *Antimicrobial Agents and Chemotherapy*, 12, 80-83

Minimum inhibitory concentrations of several antibiotics and a sulphonamide for growth of the 15 known immunotypes of *Chlamydia trachomatis* were determined in HeLa 229 cell cultures. The concentrations for complete inhibition of infectious-organism production were (per ml): tetracycline, 0.22 to 0.5 μ g; rosamycin, 0.05 to 0.25 μ g; erythromycin, 0.1 to 0.5 μ g; chloramphenicol, 10 μ g; penicillin, 0.02 to 50 u; ampicillin, 0.1 to 50 μ g; and sulphafurazole (Sulfisoxazole), 2 to 200 μ g. The same concentrations of tetracycline, rosamycin, erythromycin, and chloramphenicol were sufficient to inhibit *C. trachomatis* inclusion formation. An increased concentration of sulphafurazole was often needed to inhibit inclusion formation. Penicillin at 100 u/ml and ampicillin at 100 μ g/ml failed completely to inhibit inclusion formation.

Authors' summary

The effect of penicillin on genital strains of *Chlamydia trachomatis* in tissue culture

F. W. A. JOHNSON AND D. HOBSON (1977).
Journal of Antimicrobial Chemotherapy,
3, 49-56

The growth in McCoy cell tissue culture of strains of *Chlamydia trachomatis* recently isolated from genital infections was examined quantitatively after incubation with benzylpenicillin. An extracellular concentration of 0.1 unit/ml throughout incubation prevented the development of normal fluorescent chlamydial inclusions, but even at high concentration (100 units/ml) abnormal non-fluorescent inclusions developed. Erythromycin, chloramphenicol, and tetracycline inhibited the growth of the organisms without producing abnormal inclusions. The effect of delayed addition of penicillin or its removal during incubation was investigated. The possible nature of the abnormal inclusions resulting from exposure to penicillin is discussed.

Authors' summary

Human intra-urethral inoculation of ureaplasmas

D. TAYLOR-ROBINSON, G. W. CSONKA,
AND M. J. PRENTICE (1977).
Quarterly Journal of Medicine, 183,
309-326

Ureaplasma urealyticum was isolated from two men suffering from non-specific urethritis and from whom no other micro-organism was recovered. Their disease responded to tetracycline. The two ureaplasma strains were cloned and shown to belong to serotype 5 of *U. urealyticum*. An inoculum containing 5×10^4 colour-changing units of one strain was introduced intraurethally into a subject (an author) who was free of ureaplasmas and other urethral micro-organisms. The ureaplasmas multiplied in the urethra and the subject soon experienced symptoms of dysuria. Furthermore, polymorphonuclear leucocytes were found in centrifuged aliquots of urine and a serum metabolism-inhibition antibody response of short duration was detected. Tetracycline given six days after inoculation brought about rapid clearance of organisms from the urine and a more gradual disappearance of symptoms and signs. Inoculation of ureaplasma-free medium about two months, after the

initial introduction of ureaplasmas did not elicit symptoms or signs. These observations showed that the ureaplasmas had produced urethritis.

Organisms of the other ureaplasma strain were introduced in the same number and in the same way into a second subject (another author) who was also free of urethral micro-organisms. He experienced symptoms and signs similar to the first subject, ureaplasmas were consistently isolated from urine, and a serum antibody response of at least three months' duration was detected. However, the characteristic feature was the appearance of urinary threads composed of epithelial cells and polymorphonuclear leucocytes. Tetracycline therapy was instituted and one month after inoculation viable ureaplasmas were rapidly eliminated from the urogenital tract and, although most signs disappeared, urinary threads continued to appear. Inoculation of ureaplasma-free media during two months after the initial challenge elicited some symptoms and signs, but these were less than those which occurred after introduction of ureaplasmas. Analysis of seminal fractions during the course of the ureaplasma infection indicated that the prostate was infected. There is a possibility that the threads were of prostatic origin and the reason for their persistence is discussed.

Authors' summary

Morphology of *Ureaplasma urealyticum* (T. mycoplasma) organisms and colonies

S. RAZIN, G. K. MASOVER, M. PALANT, AND L. HAYFLICK (1977).
Journal of Bacteriology, 130, 464-511

Treatment of TRIC infection of the eye with rifampicin or chloramphenicol

J. DAROUGAR, M. VISWALINGAM, J. D. TREHARNE, J. R. KINNISON, AND B. R. JONES (1977).
British Journal of Ophthalmology, 61, 255-259

Candidosis

How often is genital yeast infection sexually transmitted?

R. N. THIN, M. LEIGHTON, AND M. J. DIXON (1977). *British Medical Journal*, 2, 93-94

Records from a clinic for sexually transmitted diseases have been used to study association between genital yeast infec-

tions and sexually transmitted diseases (STDs), non-sexually transmitted diseases (NSTDs), non-specific genital infections (NSGIs), and other conditions. Of 9094 disease episodes, 32.8% were in women, 60.5% in heterosexual men; genital yeast infections were present in 35.3% and 6.9% respectively of the episodes in each group. Yeast infection was associated with STDs and NSGIs but not with NSTDs or other conditions; they may have been sexually acquired in 39% of episodes in women and in 29% of episodes in heterosexual men. It is suggested that sexually active patients with genital yeast infections be screened for other STDs, particularly NSGI.

Tony Johnson

Detection of *Candida* precipitins by counterimmunoelectrophoresis: an adjunct in determining significant candidiasis

T. H. DEE AND M. W. RYTEL (1977).
Journal of Clinical Microbiology, 5, 453-457

Candida precipitins were determined by counterimmunoelectrophoresis (CIE) and double immunodiffusion (DID) techniques, using whole cell extracts as antigen (Hollister-Stier Labs). Sera from 164 patients were examined. Correlation between the two methods was high among 24 patients with significant *Candida* infection, and precipitin titres of 1:8 or more were detected by CIE in 17 of 22 patients with positive precipitins. Of 97 patients with either colonisation or transient infection, 50 were precipitin positive by CIE and two by DID, only three patients having a titre of 1:8. Among non-*Candida* carriers (43), 12 gave positive precipitins by CIE only. The authors concluded that quantitative estimation of *Candida* precipitins by CIE was helpful in diagnosis.

B. M. Partridge

Humoral immunity in vaginal candidiasis

S. MATHUR, G. VIRELLA, J. KOISTINEN, E. O. HORGER, T. A. MAHVI, AND H. H. FUDENBERG (1977).
Infection and Immunity, 15, 287-294

Humoral antibody titres to *Candida albicans* were estimated by the passive-haemagglutination technique. Erythrocytes were coated with protein antigen using chromic chloride as coupling agent.

Sera were obtained from 37 women with vaginal candidosis, and from 148 normal subjects (50 adult Americans and 98 Finnish blood donors). Antibody titres were higher in patients with vaginal infection, ranging from 4 to 256, compared with 0 to 16 in normal subjects. No precipitins were detected by the double-diffusion technique in sera from the infected patients. In three cases, antibodies to *C. albicans* were of the secretory immunoglobulin A type, as determined by gel filtration and double-diffusion tests, and confirmed by the indirect fluorescent antibody technique. The authors suggest that this antibody response in vaginal candidosis is mainly local.

B. M. Partridge

Comparison of cream of rice agar and horse serum for differentiating germ tubes of *Candida albicans* from filaments of *Candida tropicalis*

N. M. WARWOOD AND D. J. BLAZEVIC (1977). *Journal of Clinical Microbiology*, 5, 501-502

Genital herpes

Correlation of herpes simplex virus Types 1 and 2 with clinical features of infection

S. WOLONTIS AND S. JEANSSON (1977).
Journal of Infectious Diseases, 135, 28-33

During the course of routine diagnostic virology work, 338 strains of herpes simplex virus (HSV) were isolated in Stockholm. These strains were identified as HSV Type 1 (HSV-1) or HSV Type 2 (HSV-2) by immunoelectro-osmophoresis. This technique clearly distinguished between HSV-1 and HSV-2 and no intermediate antigenic strains were identified. The antigenic type was correlated with the age, body site, and clinical features of infection in the patients from whom HSV was recovered. Findings agreed generally with earlier studies. Thus 85% of HSV strains isolated from genital and perigenital areas were typed as HSV-2; all but one strain isolated from the mouths of patients with stomatitis or the lips of patients with herpes labialis were Type 1. Isolates from patients with keratitis or conjunctivitis, and from adults with meningoencephalitis proved to be HSV-1, whereas strains from acute aseptic

meningitis were Type 2. Five cases of neonatal HSV infections, three of them fatal, are also described. HSV-1 was isolated from three of these babies, HSV-2 from the other two.

Shirley Richmond

Occurrence of genital herpes simplex and cytomegalovirus infections in pregnancy

E. VESTERINEN, E.-R. SAVOLAINEN, E. PUROLA, E. SAKELA, AND P. LEINIKKI (1977).

Acta obstetrica et gynecologica Scandinavica, 56, 101-104

Both herpes simplex virus (HSV) infection of the cervix and cytomegalovirus (CMV) infections of pregnant women present risks to the new born infant. Infection of infants by HSV during labour may result in illnesses varying from mild to severe generalised diseases resulting in death. Severely infected infants surviving may have permanent brain damage. Intra-uterine infections with CMV commonly result in severe fetal damage resulting in microcephaly and mental retardation. The risks of infection during labour can be diminished by caesarean section but such a decision would, in the absence of obvious clinical findings, require rapid and accurate virological diagnosis. In this study a total of 244 pregnant women were screened for genital HSV and CMV infection using virus isolation, cytological examination, and serological methods. HSV could not be isolated, but CMV was found in four of the women: no abnormal smears indicative of either virus infection were seen.

Study of exfoliated cells with HSV antiserum detected immunofluorescent cells in 34 (14%) of the patients. The authors could not exclude the possibility that these reactions were non-specific and so the significance of this finding is obscure.

These results indicate that the incidence of HSV infections is low in this group of obstetric patients, considerably lower than reported by workers in the United States in studies on lower socioeconomic groups.

Serologically 14% of those studied possessed HSV antibody but the numbers did not correspond to those with immunofluorescent positive exfoliated cells; 71% possessed CMV antibodies. No differences were seen in any of the parameters tested between the first or third trimesters of pregnancy.

P. Reeve

Herpesvirus hominis Type 2 infections in asymptomatic pregnant women

R. J. BOLOGNESE, S. L. CORSON, D. A. FUCILLO, R. TRAUB, F. MODER, AND J. L. SEVER (1976).

Obstetrics and Gynecology, 48, 507-510

This study investigated the incidence of previous and current *Herpesvirus hominis* (HVH) Type 2 infections in 985 asymptomatic pregnant women. Antibodies to HVH Types 1 and 2 were determined in sera from these patients by an indirect haemagglutination test; previous HVH Type 2 infections, identified by finding a Type 2/1 antibody index of 85 or more, were found in 352 (35.7%) of these 985 women. One hundred and twenty-four (12.6%) patients had no antibodies to either HVH Type 1 or 2, and 509 (51.7%) patients had an index of <85, indicating infection with Type 1 only.

Current HVH Type 2 infections were identified by isolating the virus from 5 of 770 (0.65%) antepartum cervical specimens. No virus was isolated from 211 amniotic fluid specimens. Two patients who yielded positive cervical cultures during the second trimester underwent voluntary termination of their pregnancies. Current HVH Type 2 infections were identified in the remaining three patients late in the third trimester; two of these patients delivered vaginally and one by caesarean section. No neonatal infections were reported.

Shirley Richmond

Clinical and virological findings in patients with cytologically diagnosed gynecologic herpes simplex infections

E. VESTERINEN, E. PUROLA, E. SAKELA, AND P. LEINIKKI (1977).

Acta cytologica, 21, 199-205

Papanicolaou smears from the female genital tract, obtained from patients attending the Department of Obstetrics and Gynaecology, Helsinki University Central Hospital, were examined for evidence of herpetic infection. A cytological diagnosis was made by finding multinucleated epithelial cells with nuclear homogenisation and chromatin margination, or if intranuclear inclusions were present. Herpes simplex virus (HSV) infection was identified in 90 slides (representing 85 patients) out of a total of 57 117 (0.16%) smears examined during 1972-74.

Cytological findings in these 85 patients were correlated with recorded clinical data and with histological, virological, and serological findings when these were available. Nearly half (43%) of the cytologically-diagnosed HSV infections were asymptomatic; 15 (17%) patients had general symptoms (fever and abdominal pain), and local symptoms (genital pain, dysuria, itching, or vaginal discharge) were present in 36 (42%) cases. Ulcerative lesions were found in 17 (19%) patients.

Characteristic cytological changes occurred predominantly on the ectocervix. Viral isolation was usually successful when performed within one week of cytological diagnosis, and viral antigens could be identified in the smears by fluorescent antibody staining during this time. No morphological differences were found between primary and recurrent HSV infections.

Dysplastic changes were more frequently seen in the HSV group of smears than in a control series of smears.

Shirley Richmond

Correlation of clinical and virus-specific immune responses following levamisole therapy of recurrent herpes proctitis

R. J. O'REILLY, A. CHIBBARO, R. WILMOT, AND C. LOPEZ (1977).

Annals of the New York Academy of Sciences, 284, 161-170

Recent investigations have reported that levamisole is effective in the treatment of labial and genital *Herpesvirus hominis* (HVH) infections. It has been suggested that this antiviral agent acts by enhancing the hosts' cell-mediated immune (CMI) responses to HVH. This paper is the report of a preliminary trial of the effect of levamisole on the clinical course of recurrent genital herpes infections and on HVH-specific CMI responses, in 15 patients with culture-proved genital herpes infections, recurring every 14 to 28 days. No controls were included, and the study was not done blind.

Patients were treated with levamisole 150 mg orally on two consecutive days weekly, and were studied two weeks after initiation of treatment and monthly thereafter for between five and nine months. The clinical course, assessed by the frequency of recurrences and by the pain and duration of each, was altered favourably in eight of 12 patients. Three patients reported no change, and one

- patient reported increased frequency of infections. CMI responses, assessed by lymphocyte transformation and assay of leucocyte migration inhibitory factor to HVH antigen, were enhanced in six of the eight patients who reported fewer recurrences. In the four patients with no clinical improvement, CMI responses were depressed or unchanged. Alterations in HVH-specific CMI responses therefore correlated with changes in the clinical course of the infections.

Shirley Richmond

Effect of ribavirin on Type 2 *Herpesvirus hominis* (HVH/2) *in vitro* and *in vivo*

L. B. ALLEN, S. M. WOLF, C. J. HINTZ, J. H. HUFFMAN, AND R. W. SIDWELL (1977). *Annals of the New York Academy of Sciences*, 284, 247-253

The effect of ribavirin (Virazole, ICN 1229), a synthetic antiviral compound, on the growth of Type 2 *Herpesvirus hominis* (HVH/2) was assessed in *in vitro* cell cultures and in experimentally induced tail and genital HVH/2 lesions in mice. Ribavirin inhibited the growth of five strains of HVH/2 tested in KB cell monolayers; there were strain differences in the sensitivity of ribavirin, the overall MIC range being 10-100 µg/ml. In the experimentally infected mice topical ribavirin reduced both tail and genital lesions induced by a single strain (VF-2) of HVH/2. Since intravaginal treatment with ribavirin initiated late in the infection was effective, this compound may have potential for treatment of human cutaneous infections of HVH/2.

Shirley Richmond

Simultaneous isolation of *Herpesvirus hominis* Type 2 and cytomegalovirus from the genital tract of a woman

F. R. MANUEL AND J. A. EMBIL (1977). *Sexually Transmitted Diseases*, 4, 18-19

Herpes simplex virus Type 2 meningitis (Case Report)

J. E. HEVRON JR (1977). *Obstetrics and Gynecology*, 49, 622-624

Other sexually transmitted diseases

Lymphogranuloma venereum presenting as superclavicular and inguinal lymphadenopathy

P. D. WALZER AND D. ARMSTRONG (1977). *Sexually Transmitted Diseases*, 4, 12-14

Miscellaneous

Vaginal colonisation with group B *Streptococcus*: a study in college women

C. J. BAKER, D. K. GOROFF, S. ALPERT, V. A. CROCKETT, S. H. ZINNER, J. R. EVRARD, B. ROSNER, AND W. M. MCCORMACK (1977). *Journal of Infectious Diseases*, 135, 2-397

The group B *Streptococcus* is now recognised as a common neonatal pathogen and is generally transmitted intrapartum from the maternal genital tract. Vaginal specimens were obtained from 499 college students. Group B *Streptococci* were isolated from 18%. Serotype III (37.9%) and II (25.3%) were particularly common. The factors which aided colonisation were the presence of an intrauterine device, sexual experience, age under 20, and during the first half of the menstrual cycle. Several other factors including a history of venereal disease were unrelated to colonisation. It appears that sexual experience and possibly host defence mechanisms which are age-related, were among the most important positively correlated factors.

G. W. Csonka

Isolation of *Neisseria meningitidis* in urogenital/rectal infections

K. ØDEGAARD AND T. GUNDERSEN (1977). *Acta dermato-venereologica*, 57, 173-176

During 1975, *N. meningitidis* was isolated on 10 occasions from samples taken from urogenital and rectal sites in nine patients. Of the meningococci isolated, one belonged to serogroup A, six to group B, and two to group C. A total of 90 540 samples had been cultured for gonococci and 7045 had been positive.

Six patients (two women and four men) who had attended the Department of Venereal Diseases, Oslo, are described in detail. One woman had a slight vaginal discharge and meningococci were isolated from the cervix; both the meningococci and the symptoms cleared without treatment. The other woman had symptomless urethral meningococci which had disappeared 14 days later on repeat culture. Two of the four men were homosexual. Three had symptomless urethral meningococci which were absent at follow-up without treatment. The fourth man had

symptomless rectal meningococci which could still be isolated 14 days later; he was considered to be a symptomless carrier.

D. H. Jackson

Patterns of sexually transmitted enteric diseases in a city

T. E. AINSWORTH, A. BACK, L. A. BOUCHER, W. F. GARRARD, R. D. PALMER, AND E. RIVER (1977). *Lancet*, 2, 3-4

In the past three years cases of shigellosis, amoebiasis, and viral hepatitis A and B have increased fourfold to tenfold in San Francisco. These diseases were particularly common in people who had adopted 'alternative life-styles', especially young men. In many cases there was a history of frequent orogenital and oral-anal sexual contact between men with no common food source. Despite energetic public health measures and intensive efforts by physicians treating these cases, the increase has not yet been brought under control. Other cities may experience similar difficulties with sexual transmission of enteric diseases.

Authors' summary

Quantitative bacteriology of the vaginal flora

J. G. BARTLETT, A. B. ONDERDONK, E. DRUDE, C. GOLDSTEIN, M. ANDERKA, S. ALPERT, AND W. M. MCCORMACK (1977). *Journal of Infectious Diseases*, 136, 271-277

Quantitative bacteriology was performed on vaginal secretions from healthy adult women. The analysis included a single sample from 17 college students and 35 samples from five volunteers collected at intervals of three to five days throughout the menstrual cycle. Mean concentrations in all 52 specimens were $10^{5.1}$ aerobic bacteria/g and $10^{9.1}$ anaerobic bacteria/g. The rank of predominant organisms, according to rates of recovery in concentrations of $>10^5$ colony-forming units/g, was anaerobic and facultative *Lactobacillus* species, *Peptococcus* species, *Bacteroides* species, *Staphylococcus epidermidis*, *Corynebacterium* species, *Peptostreptococcus* species, and *Eubacterium* species. Sequential samples collected throughout the menstrual cycle showed relatively consistent mean levels of anaerobes and a significant decrease in

concentrations of aerobes in premenstrual specimens compared with those in the specimens collected in the week following onset of menses. Analysis of sequential specimens from each of the five individuals showed considerable variation in species recovered. These data indicate that the vaginal flora in healthy adult women is a dynamic ecosystem in which anaerobes are usually the numerically dominant bacteria.

Authors' summary

The condom and gonorrhoea

D. BARLOW (1977). *Lancet*, 2, 811-812

The use of the condom by male patients attending a clinic specialising in sexually transmitted diseases has been assessed

over a period of six months. Condom users were divided into those who used them properly and invariably, and those who did not. In the group studied, correct use of the condom was associated with a significantly lower probability of acquiring gonorrhoea ($P < 0.001$) and a significantly higher chance of there being no sexually transmitted disease diagnosed ($P < 0.0005$). The diagnosis rate of non-specific urethritis, however, did not differ among the groups.

Author's summary

Inhibition of *Corynebacterium vaginale* by metronidazole

R. F. SMITH AND W. E. DUNKELBERG (1977). *Sexually Transmitted Diseases*, 4, 20-21

Tellurite reduction test to aid in the recognition of *Corynebacterium vaginale*
R. F. SMITH, J. L. VOSS, AND R. K. BAILEY (1977). *Journal of Clinical Microbiology*, 5, 375-380

The expanding spectrum of Behçet's syndrome

E. KANSU, S. DEGLIN, R. I. CANTOR, J. F. BURKE, S. Y. CHO, AND R. T. CATHART (1977). *Sexually Transmitted Diseases*, 237, 1855-1856

Treatment of balanitis xerotica obliterans with testosterone propionate ointment

T. A. H. PASIECZNY (1977). *Acta dermato-venereologica*, 57, 275

Book review

Burckhardt's Atlas and Manual of Dermatology and Venereology. Third American Edition. Edited by P. J. Lynch and S. Epstein. 1977. Pp. 271, 178 figures (including 111 in colour). Williams and Wilkins, Baltimore, USA (£26.00)

The first half of this book gives a brief account of the various skin diseases, including their treatment. Venereal diseases are devoted a chapter of eight pages and other diseases of the genitalia three pages. The preface states 'this was to be a short and simple, but nevertheless

inclusive, introduction to dermatology and venereology for those who are not dermatologists'. This certainly does not apply to venereology. There are a total of six illustrations of genital lesions. Among the 18 illustrations of syphilitic lesions there are two rather similar ones of secondary palmar syphilides and one showing bismuth lines of the gums, surely now of only historical interest. The space would have been better used to provide a clinical illustration of scabies. In 1977 one might have expected an illustration of the skin lesions of gonococcal septicaemia.

The only figure relating to gonorrhoea is one showing Gram-negative diplococci in black and white! There are some misleading statements—for example, that Hutchinson's teeth are 'notched central incisors'. The index gives page references to the figures but the pages of the section containing the figures are not numbered!

Anyone wanting an introduction to venereology will be disappointed and will certainly not want to pay £26.00 for this book.

P. Rodin

Notice

Dr R. D. Catterall has been appointed Adviser in Genitourinary Medicine to the Chief Medical Officer of the Department of Health and Social Security from 1 January 1978. He succeeds Dr C. S. Nicol who has retired.

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Contents

Editorial: clinic practices *page 1*

Facilities and diagnostic criteria in sexually transmitted disease clinics in England and Wales
M. W. ADLER, E. M. BELSEY, B. H. O'CONNOR, R. D. CATTERALL, AND D. L. MILLER (Gt Britain) *page 2*

Diagnostic treatment and reporting criteria for gonorrhoea in sexually transmitted disease clinics in England and Wales: 1. Diagnosis
M. W. ADLER (Gt Britain) *page 10*

Diagnostic treatment and reporting criteria for gonorrhoea in sexually transmitted disease clinics in England and Wales: 2. Treatment and reporting criteria
M. W. ADLER (Gt Britain) *page 15*

Sexually transmitted diseases: the present situation in Sweden
J. WALLIN (Sweden) *page 24*

Epidemiology and treatment of gonorrhoea caused by penicillinase-producing strains in Liverpool
O. P. ARYA, E. REES, A. PERCIVAL, C. D. ALERGANT, E. H. ANNELS, AND G. C. TURNER (Gt Britain) *page 28*

Cultural diagnosis of gonorrhoea with modified New York City (MNYC) medium
H. YOUNG (Gt Britain) *page 36*

Isolation of *Neisseria meningitidis* and *Neisseria catarrhalis* from the genitourinary tract and anal canal
C. BLACKWELL, H. YOUNG, AND S. S. R. BAIN (Gt Britain) *page 41*

Chlamydial infection of the urethra in men
H. M. PERROUD AND K. MIEDZBYBRODZKA (Switzerland) *page 45*

A trial of adenine arabinoside in genital herpes
A. L. HILTON, T. E. C. BUSHELL, D. WALLER, AND J. BLIGHT (Gt Britain) *page 50*

Circulating immune complexes in syphilitic nephropathy: a case report
J. SØLLING, K. SØLLING, K. U. JACOBSEN, S. OLSEN, AND E. FROM (Denmark) *page 53*

Report of the Chief Medical Officer for 1976 *page 57*

Abstracts *page 60*

Book review *page 68*

Notice *page 68*

7778
April 1978 Vol. 54 No. 2

British Journal of
Venereal Diseases

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Evans, B. A. (1976). Detection of gonorrhoea in women. *British Journal of Venereal Diseases*, 52, 40-42.

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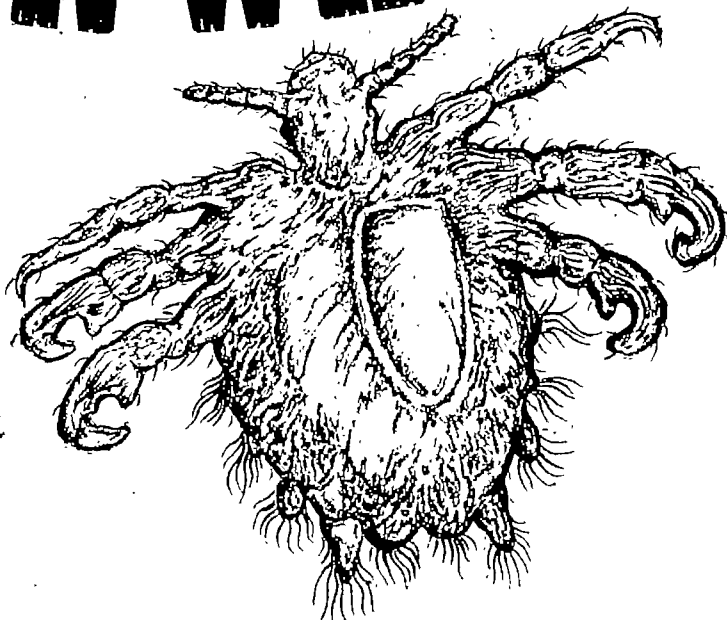
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Editorial

The nitroimidazole family of drugs

In 1955 an antibiotic complex isolated from a strain of *Streptomyces* on the island of Reunion was found by research workers of Rhône-Poulenc in Paris to contain a trichomonocidal antibiotic—azomycin. It had previously been isolated in Japan (Maeda *et al.*, 1953) and identified as 2-nitroimidazole (Ia see Table) (Nakamura, 1955). At the time, and for some years after, this remarkably simple compound defied synthesis, but it stimulated the workers at Rhône-Poulenc to prepare and test the activity of the more readily accessible isomeric 5-nitroimidazoles (II). It was their good fortune in 1957 to find that these isomers were more active antiprotozoal agents than the natural product (Cosar and Julou, 1959). In a series of 150 related compounds, the one with a β -hydroxyethyl group in the 1-position gave the best compromise between activity and toxicity and this brand of metronidazole was introduced as Flagyl.

By the end of the decade, metronidazole was well established as the first agent systemically effective against *Trichomonas vaginalis* and *Trichomonas foetus*. In this subsequent period other protozoa—such as, *Giardia lamblia* and *Entamoeba histolytica*—which share with trichomonad species a parallel sensitivity to various drugs, were shown to be susceptible. Through the 1960s the potential of the drug as treatment for the serious tropical disease amoebiasis was explored and established in both the intestinal and extra-intestinal forms, including symptomless cyst passers.

During this period, many additional therapeutic claims were made in an astonishing variety of conditions, ranging from alcoholism to haemorrhoids, but of the few that stood the test of time the most significant was the treatment of acute ulcerative gingivitis, Vincent's angina, after acute clinical observation by a British dental surgeon (Shinn, 1962).

A much more recent development, which followed the recognition of the importance of certain groups of non-sporing anaerobic bacteria and especially *Bacteroides fragilis* in a wide range of infections, has been the establishment of metronidazole as a specific highly effective drug for the treatment of these infections. It is also useful in preventing post-

operative infection caused by susceptible anaerobes, particularly in gynaecological surgery, appendectomy, and colonic surgery.

Real innovations in chemotherapy, such as metronidazole, always attract attention from other research groups. Although interest was slow to develop, research workers have sought analogous, structurally-modified compounds which might afford some advantage in clinical use—for example, greater potency, better tolerance and freedom from side effects, a broader spectrum of action, a longer duration of action, or in some other characteristic. This effort has been concerned with important veterinary uses of 5-nitroimidazoles as well as the applications in human medicine.

Metronidazole has been a difficult target to improve upon, but several other drugs of this chemical family have been introduced to clinical practice and many more have been described in various stages of research. The main purpose of this editorial is briefly to review these newer drugs and their relationships.

It is worth noting that the first direct challenge to metronidazole as a systemically effective trichomonocide was not a 5-nitroimidazole. The Italian discovery nifuratel (Magmilor), marketed in the United Kingdom in 1960, is a nitrofurantoin, a series that includes such drugs as nitrofurantoin and nitrofurazone (but, as such, is outside the scope of this article).

The second nitroimidazole to be introduced was nimorazole (IIb) (Naxogin), previously known as nitrimidazine. This was also discovered in Italy, by the research workers of Carlo Erba (de Carneri *et al.*, 1969), and it was introduced in Britain in 1970. By the middle of 1977 no other member of this class had been marketed in this country for use in human medicine. Two others are however in use on the Continent, these being tinidazole (IIc) (Fasigyn, Pfizer) (Miller *et al.*, 1969), which has been available for about six years, and ornidazole (IId) (Tiberal, Hoffmann-La Roche), a recent introduction. Three other products which have attracted recent interest are secnidazole (IIe) (see page 77), carnidazole (IIff), and a 2-nitroimidazole, Ro 07-0582 or misonidazole (Ib), which has been

the subject of recent investigations in a different field, that of radiosensitisation.

The close structural relationships of these compounds are demonstrated in the Table, which shows that the above products, apart from misonidazole, differ only in the groups attached to the ring nitrogen adjacent to the nitro group. Secnidazole is a simple homologue of metronidazole; ornidazole differs from it only by having a hydrogen replaced by chlorine. These modest changes in structure, as well as the basic substituent, morpholine, in nimorazole, the sulphone group in tinidazole, and the thiocarbamate system in carnidazole may be expected to modify various physicochemical and biochemical characteristics—such as, distribution between aqueous and lipid media, transport across membranes, metabolic pathways, tissue distribution, routes of excretion, and other properties. They probably have little effect on the essential anti-protozoal or antibacterial action, which appears to be a function of the nitro group associated with the imidazole ring.

Comparisons of the anti-infective activity of related drugs are notoriously misleading when they are based on results from different laboratories using different strains of pathogen and different experimental techniques. Broad generalisations alone are possible from a review of the literature. The first is that *in vitro* activities of the five 5-nitro drugs against *T. vaginalis* or *T. foetus* are of the same order, and minimal inhibitory concentrations range from 0.12 to 6.0 µg/ml. There are minor variations in activity against *G. lamblia* and *E. histolytica*, metronidazole being among the most potent; and somewhat more variation against Gram-negative anaerobic bacteria, where nimorazole is slightly less active than the others.

The comparative trichomonocidal activity of these drugs in experimental animals differs with the host and infecting species used; ornidazole has an ED₅₀ of 3 mg/kg against *T. foetus* but 37 mg/kg against *T. vaginalis* in the mouse (Grunberg *et al.*, 1970). It has been shown in some reports that nimorazole and tinidazole have lower ED₅₀s than metronidazole but other reports have not substantiated this. It is doubtful whether differences in the values are often of much therapeutic significance.

All these drugs are absorbed efficiently from the digestive tract after oral administration, but they differ in their pharmacokinetic properties in humans. The serum half-life of tinidazole (12.5 h) is nearly twice that for metronidazole (7.3 h), while that of secnidazole (17 h) is half as long again. Nimorazole is more rapidly absorbed and excreted than metronidazole and it may be some advantage that its two main urinary metabolites retain antiprotozoal activity. It is also claimed that this compound is more effectively absorbed from pessaries (de Carneri *et al.*, 1969).

Are these modest differences of practical relevance in clinical practice? Again it is not easy to draw clear-cut conclusions by a review of published work. Cure rates for trichomoniasis are usually good (85 to 95%) but they are complicated by the number of relapses and by whether or not these are re-infections: the concomitant treatment of consorts always gives better results. When studies on metronidazole and on each of the other drugs have been compared, using similar dosage schedules, the results have been similar.

The subject of dosage schedules has received much attention, with shorter schedules being sought for the newer drugs which would be an advantage in the type of patient often seeking treatment.

Table Structures of nitroimidazoles studied in human medicine

(I)	(II)
(Ia) R ¹ = H	(IIa) R ¹ = -CH ₂ CH ₂ OH; R ² = CH ₃
(Ib) R ¹ = -CH ₂ OH CH ₂ CH ₂ OCH ₃ misonidazole	(IIb) R ¹ = -CH ₂ CH ₂ N O nimorazole
	(IIc) R ¹ = -CH ₂ CH ₂ SO ₂ CH ₂ CH ₃ ; R ² = CH ₃ tinidazole
	(IId) R ¹ = -CH ₂ OH CH ₂ CH ₂ Cl; R ² = CH ₃ ornidazole
	(IIe) R ¹ = -CH ₂ OH CH ₂ CH ₃ ; R ² = CH ₃ secnidazole
	(IIIf) R ¹ = -CH ₂ CH ₂ NH-CS-OCH ₃ ; R ² = CH ₃ carnidazole

Whereas the standard course originally established for metronidazole was 200 mg thrice daily for seven days, nimorazole was introduced in regimens of three 1 g doses at 12-hourly intervals or even as a single 2 g dose. Metronidazole has since been shown to be effective in these regimens.

The single-dose treatment has obvious clinical attractions and there is evidence that all five drugs give a good cure rate after such a schedule. Ornidazole has been shown to give successful cures in 87% of cases after a single course of four 500 mg pessaries (Hillström *et al.*, 1977; Sköld *et al.*, 1977). The high success rate after a single 2 g dose of secnidazole is reported, see page 77, but it is perhaps too early to say the longer the half-life in serum the higher the cure rate after a single dose. The only information on carnidazole is that given by Notowicz *et al.* (1977) who showed that a high cure rate was achieved in women with a single 2 g dose.

Resistance to metronidazole among trichomonads has been induced in laboratory experiments but it has very rarely, if ever, been responsible for therapeutic failure of which the commonest cause is reinfection. Cross-resistance in trichomonads is shown in the laboratory between nitroimidazoles, as might be expected between drugs probably acting by the same mechanism, but it may be incomplete.

Studies of the newer products in amoebiasis have been limited and conclusions must be tentative. Powell and Elsdon-Dew (1972), of the amoebiasis research unit in Durban, compared four of these drugs in the treatment of amoebic liver abscess. Ornidazole came nearest to the performance of metronidazole, but tinidazole was slower in action, and nimorazole failed to eradicate the intestinal infection, as shown by the number of cyst passers after treatment. Insufficient activity in the gut is a weakness of all the 5-nitroimidazoles. Indian workers however obtained equal results with metronidazole and tinidazole when they compared five-day courses of 400 mg three times a day of the former with 600 mg twice a day of the latter (Misra and Laig, 1974; Prakash *et al.*, 1974).

In clinical reports, the effectiveness of metronidazole against Gram-negative anaerobic infections is well established. While there is no reason to doubt the potential efficacy of the newer drugs, clinical evidence has not yet been published.

The 2-nitroimidazole, misonidazole, also developed by Hoffmann-La Roche, is of interest in a different connection. In 1973, Dr R. L. Willson of Brunel University demonstrated that metronidazole, probably because of its marked electron-affinity character, was able to render hypoxic tumour cells more sensitive to radiotherapy (Foster and Willson,

1973). This has been confirmed by clinical trials at Mount Vernon Hospital, Northwood (Deutsch *et al.*, 1975) and also by North American investigators (Urtasun *et al.*, 1975, 1976). In a search for related compounds with higher electron-affinity, 2-nitroimidazoles were found to be promising and misonidazole was selected for its high potency. Unfortunately the doses used were still very large: this experimental drug shows signs of being too toxic for regular use for this radiosensitising purpose.

In this special field therefore there is much scope for a more satisfactory compound; in the traditional fields significant improvement on existing agents will probably be extremely difficult to attain.

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Studies on strain sensitivity of *Trichomonas vaginalis* to metronidazole

J. G. MEINGASSNER, LISELOTTE HAVELEC, AND H. MIETH

From Sandoz Forschungsinstitut and Institut für Medizinische Statistik und Dokumentation der Universität, Vienna, Austria

SUMMARY Ninety-four strains of *Trichomonas vaginalis* isolated from unselected patients in Vienna during the period from November 1974 to November 1975 were subjected to sensitivity tests against metronidazole *in vitro* and *in vivo*. Nearly 90% of the isolates examined showed *in vitro* growth inhibition at concentrations of 0.4 to 1.6 µg metronidazole/ml and only about 10% at concentrations of less than 0.4 µg/ml. All isolates showed some inhibition of growth after incubation for 48 hours with 3.2 µg metronidazole/ml. Tests on treatment carried out on experimentally infected mice revealed that all strains of *T. vaginalis* examined were sensitive to metronidazole (MDE: <3 × 35 mg/kg peroral).

Introduction

Reports on treatment failures with metronidazole (Robinson, 1962; de Carneri *et al.*, 1963; Arnold, 1966; de Carneri, 1966; Diddle, 1967; Aure and Gjønnaess, 1969; Kurnatowska, 1969; Giannone, 1972) have been the subject of much discussion and a possible drug resistance of *T. vaginalis* has been questioned (Jennison *et al.*, 1961; Kane *et al.*, 1961; Squires and McFadzean, 1962; Nicol *et al.*, 1966b; McFadzean *et al.*, 1969; Keighley, 1971; Korner and Jensen, 1976). These observations vary primarily because of divergent findings on the susceptibility of isolates from so-called 'therapeutic non-starters' to metronidazole as demonstrated by Jennison *et al.* (1961), Robinson (1962), de Carneri *et al.* (1963), Nicol *et al.* (1966b), Kurnatowska (1969), and McFadzean *et al.* (1969).

However, experimental studies have revealed that trichomonad strains whose resistance to metronidazole was proved by tests on treatment in laboratory animals, hardly differed from sensitive reference strains when they were tested for *in vitro* sensitivity (de Carneri *et al.*, 1969; Benazet and Guillaume, 1971). Thus, the value of such sensitivity tests appears to be in doubt (de Carneri *et al.*, 1969). A possible explanation for these observations has recently been found in our investigations (Meingassner and Lindmark, 1977): it was shown that a strain of *Trichomonas foetus* (KV₁/M100), the causative agent of urogenital trichomoniasis in cattle, developed resistance to metronidazole (Meingassner and Mieth, 1976) in mice after several

syringe passages under increasing drug concentrations. However, as with strains of *T. vaginalis* showing *in vivo* resistance to metronidazole, this strain exhibited the same sensitivity to metronidazole *in vitro* as the sensitive parent strain when examined under anaerobic test conditions. Under aerobic test conditions the KV₁/M100 strain also proved to be highly resistant to metronidazole.

In response to these findings we have tested unselected isolates of *T. vaginalis* for their sensitivity to metronidazole in laboratory animals as well as under aerobic test conditions *in vitro* in order to ascertain the range of variation of the sensitivity of *T. vaginalis*.

Material and methods

ISOLATION AND CULTURE OF *T. VAGINALIS*

Vaginal swabs were collected from 100 untreated patients with symptomatic trichomoniasis. The swabs were rinsed in a trichomonas medium (Oxoid, CM 161) containing antibiotics and the medium was then incubated for three days. In order to eliminate the concomitant microbial flora subpassages were made with 1000 iu of penicillin, 1000 µg of streptomycin, and 10 iu of nystatin per ml medium. Eventually 94 isolates were cultivated under axenic conditions. Determination of the sensitivity was made on these cultures within 12

Address for reprints: Dr J. G. Meingassner, Sandoz Forschungsinstitut, Brunnerstrasse 59, A-1235 Wien, Austria

Received for publication 23 August 1977

passages. All strains subjected to the sensitivity test had been isolated in Vienna during the period from November 1974 to November 1975.

EVALUATION OF THE *IN VITRO* SENSITIVITY

The sensitivity of each isolate to metronidazole was estimated by determining the minimal inhibitory concentration (MIC) and the minimal lethal concentration (MLC) in a serial dilution test.

BROTH DILUTION TEST PROCEDURE

The cups of sterile cell-culture plates (Linbro Chemical Co. Inc., New Haven, Conn./USA, model 96 CV-TC) were filled with 1.4 ml of the diluted overnight cultures of the isolates (final concentration about 50 000 trichomonads/ml CM 161-medium) and with 0.1 ml of metronidazole solution. The plates were then sealed with a pressure-sensitive tape (Scotch) and incubated for 48 hours at 37°C. The evaluation of each isolate was made in one test with two replications. The concentration (using a twofold dilution schedule) ranged between 0.05 and 100 µg of metronidazole/ml.

In order to determine the MIC and MLC the plates were examined in an inverted microscope with a 40× magnification. The growth was estimated subjectively in accordance with the system + to +++, whereby the lowest concentration with + growth was regarded as the MIC. (Single motile trichomonads per cup were read as + and the culture density corresponding to the growth in untreated controls as +++). The MLC was the lowest concentration in which no motile trichomonads could be detected. The final evaluation was the result of the majority of the individual findings. A sensitive strain of *T. vaginalis* (A, isolated in 1971) was used as a control in every test.

EVALUATION OF THE *IN VIVO* SENSITIVITY

The chemotherapeutic sensitivity of the isolates was determined on ectopically-infected mice which were checked for the presence or absence of lesions and parasites six days postinfection after three peroral treatments.

IN VIVO TESTING PROCEDURE

Female NMRI-mice weighing 10–12 g were treated orally with metronidazole 2, 18, and 24 hours after a double infection with 1.2×10^8 trichomonads intraperitoneally and 4×10^8 trichomonads subcutaneously. For the isolates nos 1–70 dosage schemes of 3×15 , 25, and 35 mg of metronidazole/kg body weight were applied using six mice per concentration, while only one dose of 3×25 mg/kg on 12 mice per concentration was used for isolates

nos 71–94, as it had been found that all these dosage schemes were statistically equally reliable.

The animals were killed on the sixth day after infection. The criteria of antitrichomonal efficacy of metronidazole were based on the absence of macroscopically visible lesions in the subcutis (abscesses) and on the absence of motile trichomonads in 48-hour cultures of the abdominal cavity. To compare the dose responses the score system shown in Table 1 was used:

Table 1 Score system

Score	Culture density/abdomen	Size of abscesses/subcutis	
		Description	Approximate size (mm)
0	0	Normal	
1	(+)	Pinhead	2–3
2	+	Lentil	4
3	++	Pea	5–6
4	+++	Bean	>6

As a control, infected, untreated groups were used to determine the pathogenicity of the isolates for mice, as well as treated groups infected with a reference strain (E, isolated in 1971) to compare the sensitivity.

Smears from abscesses were examined microscopically for motile trichomonads.

STATISTICAL TREATMENT

The total frequency distribution of the MIC and MLC of the 94 isolates was compared with that of the control strain A, estimated by means of the χ^2 test. This test was also applied for assessing the metronidazole efficacy *in vivo*, compared with the reference strain E.

Results

The *in vitro* metronidazole sensitivity of all isolates tested is summarised in Figs 1 and 2. Growth inhibition was achieved in nearly 90% with concentrations ranging from 0.4 to 1.6 µg metronidazole/ml medium, and in about 10% with concentrations of less than 0.4 µg/ml. All isolates showed an inhibited growth *in vitro* after an incubation period of 48 hours with 3.2 µg of metronidazole/ml (Fig. 1). The values of the MLC showed a similar variation in metronidazole sensitivity. Concentrations ranging from 1.6 to 3.2 µg were lethal in 85% of the isolates whereas lower concentrations were trichomonacidal in only 15%. In a single isolate—although its MIC did not differ from those of the remaining isolates—the lethal concentration was found to be >3.2 µg (Fig. 2). No marked differences in metronidazole

sensitivity could be found between the isolates tested and the reference strain after 14 separate tests.

The findings of the sensitivity tests *in vivo* are summarised in Tables 2 and 3. The results of treatment in ectopically infected mice showed that all strains had the same degree of sensitivity to metronidazole. The total healing rate ascertained with doses of 25 mg metronidazole/kg body weight was 81.7% by the use of the pathological findings and 78.4% according to the results of reisolation. Not more than 33.3% of mice per treatment group remained infected after treatment with 3×25 mg

metronidazole/kg. The result for the groups treated with dosages of 3×35 mg/kg body weight was 99% (Table 2). The infected untreated controls showed that all isolates were pathogenic for mice.

The sensitivity of the isolates obtained from the ectopic infection model *in vivo* did not differ significantly from that of the reference strain E.

A comparative analysis of the efficacy of metronidazole at the two sites of infection is presented in Table 3.

In 77.6% of mice treated with 3×25 mg metronidazole/kg body weight both infection sites were cured. In 17.7% the subcutaneous infection was

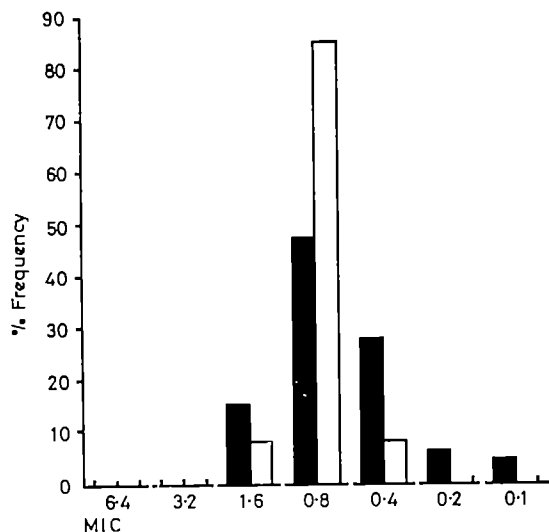


Fig. 1 In vitro activity of metronidazole against 94 isolates and a reference strain of *T. vaginalis*: minimal inhibitory concentration (MIC) in $\mu\text{g/ml}$.

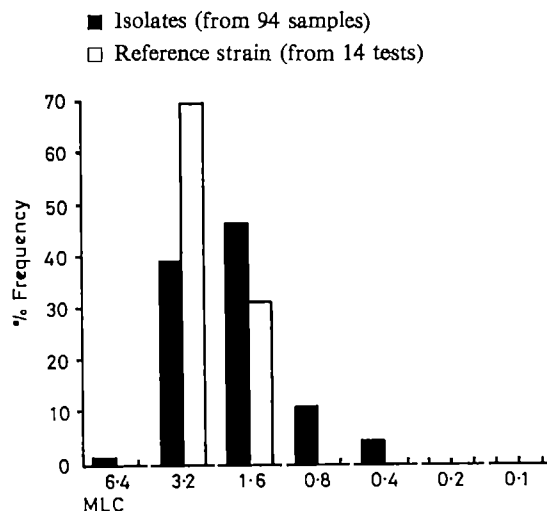


Fig. 2 In vitro activity of metronidazole against 94 isolates and a reference strain of *T. vaginalis*: minimal lethal concentrations (MLC) in $\mu\text{g/ml}$.

Table 2 Efficacy of metronidazole against 94 isolates and a reference strain of *T. vaginalis* in mice: incidence of the individual findings scored with 0-4 after peroral treatment

Samples of <i>T. vaginalis</i>	Dosage (mg/kg body weight)	Score (% incidence)					No. of animals
		0	1	2	3	4	
Isolates	3×15	5.0	69.2	23.5	2.3	0.0*	302†
		1.7	3.6	38.1	54.6	2.0†	
	3×25	81.7	17.8	0.3	0.2	0.0*	870
		78.4	7.1	13.3	1.3	0.0†	
	3×35	99.1	0.9	0.0	0.0	0.0*	325‡
		99.4	0.3	0.3	0.0	0.0†	
Infected controls	Untreated	0.0	10.5	52.0	37.5	0.0*	564
		0.0	0.0	0.0	51.6	48.4†	
Reference strain E	3×25	88.6	11.4	0.0	0.0	0.0*	184‡
		81.5	3.8	14.7	0.0	0.0†	

* Lesions in the subcutis

† Density of trichomonads in cultures of the abdominal cavity

‡ Several groups comprised fewer than 6 or 12 animals

Table 3 Comparison of the efficacy of metronidazole in mice according to the mode of infection: incidence of different findings after peroral treatment of subcutaneous or intraperitoneal infections

Samples of <i>T. vaginalis</i>	Dosage (mg/kg body weight)	Differences in the scoring system (% frequency)			No. of animals
		Intraperitoneal < subcutaneous*	Intraperitoneal = subcutaneous†	Intraperitoneal > subcutaneous‡	
Isolates	3 × 15	89	9.3	1.7	302§
	3 × 25	17.7	77.6	4.7	870
	3 × 35	0.6	98.5	0.9	325§
Reference strain E	3 × 25	18.5	80.4	1.1	184§

*Clearance better in subcutaneous infections than in intraperitoneal infections

†Clearance identical in subcutaneous and intraperitoneal infections

‡Clearance better in intraperitoneal infections than in subcutaneous infections

§Several groups comprised fewer than 6 or 12 animals

cleared and the peritoneal was not, whereas the reverse was true in only 4.7%.

Discussion

In vitro sensitivity tests carried out on 94 isolates of *T. vaginalis* revealed that all specimens were sensitive to metronidazole to almost the same degree. This was also shown very clearly by the results from the experiments in mice. The MLC value of only one strain was 6.4 µg/ml, although its growth sensitivity and susceptibility to metronidazole *in vivo* did not differ from the remaining isolates. The reason we employed the double-infection method was that while subcutaneous infections with strains of *T. vaginalis* of unknown virulence can more easily be established than intraperitoneal infections, intraperitoneal infections do permit a more reliable evaluation of treatment. However, in our assay all intraperitoneal infections proved to be successful. Distinct therapeutic differences owing to the mode of infection were found in the dosage group of 3 × 15 mg/kg which, we believe, might be explained by the different modes of assessment used. The results of treatment in mice infected subcutaneously were assessed macroscopically (which may be considered a rather rough evaluation), while intraperitoneally infected mice were evaluated by reisolating trichomonads from the abdominal cavity which proved to be far more reliable.

Various workers have pointed out (Nicol *et al.*, 1966a; McFadzean *et al.*, 1969) that it is possible that metronidazole may be inactivated by the vaginal bacterial flora, so we used only decontaminated cultures for our studies. Those samples which could not be cultivated axenically with penicillin, streptomycin, and nystatin after 12 subpassages were therefore rejected.

Considering the fact that we conducted our studies on unselected patients suffering from vaginal discharge and it may therefore be assumed

that they comprised primary cases and reinfections as well as exacerbations or uncured cases (although no amnestic details were known), we feel that the results were remarkably uniform. In neither test system did the isolates significantly differ one from another or from the reference strains regarding their sensitivity. Resistant and 'relatively' resistant strains were not found with our assay system, nor did the strains show a gradual decrease of susceptibility compared with the reference strains as described by Giannone (1972).

We wish to thank Dr O. Palla and Dr J. Scholz for providing the test strains, Mr K. Stockinger for his technical assistance, and Dr P. F. Troke for his criticism of the manuscript.

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Secnidazole

A 5-nitroimidazole derivative with a long half-life

D. VIDEAU,* G. NIEL,* A. SIBOULET,† AND F. CATALAN‡

From the *Institut de Biopharmacie, Rhône-Poulenc Santé, Paris, †Hôpital St-Louis, Paris, and ‡Institut Alfred-Fournier, Paris

SUMMARY The therapeutic activity of a single 2 g dose of secnidazole was studied in patients with urogenital trichomoniasis. In 140 patients, 97% were cured and the drug was well tolerated. In the laboratory, tests on sensitivity were made and the minimal inhibitory concentration (MIC) and the minimal trichomonocidal concentration (MTC) were determined on cultures that had recently been isolated at the clinic, and the pharmacokinetic properties of secnidazole in man were compared with those of tinidazole. The therapeutic efficacy of all the metronidazole derivatives was reviewed and a single-dose treatment is recommended. Therapeutic and prophylactic treatment is achieved by products with a long half-life. Secnidazole, with a half-life of 14.3 ± 1.3 h (women) and 20.2 ± 3.1 h (men), is particularly suitable for this type of treatment.

Introduction

Urogenital trichomoniasis is a common sexually transmitted disease (STD). Recent studies by French workers in Paris, Corsica, and Senegal showed that 20 to 25% of women and 15% of men who have a non-gonococcal genital infection are infected by this parasite (Nicoli *et al.*, 1964; Ridet and Siboulet, 1974-75, unpublished; Bohbot, 1975).

As a result of this work and because of the current epidemiological need for a single-dose treatment (treatment 'within a minute') (Siboulet and Durel, 1961; Csonka, 1971), the interest of clinicians has been focused on new products.

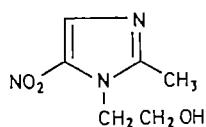
Two studies, one clinical and one laboratory, have been carried out in parallel on a new derivative of 5-nitroimidazole—14-539 RP or secnidazole. In the laboratory study, the biological activity and pharmacokinetic properties of secnidazole were compared with those of metronidazole and tinidazole. The half-life of secnidazole made it especially interesting, particularly for the type of treatment stated above.

Patients and methods

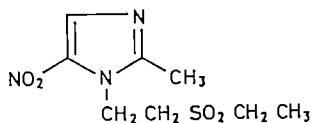
One hundred and forty patients (56 men and 84 women) aged between 15 and 54 years, were treated at two centres with one 2 g dose of secnidazole (4×500 mg tablets). Pregnant women were not included in the trial. To complete the study a larger dose (2.5 g) was given to 110 patients to find out if this improved the rate of cure. Patients in the second group matched patients in the first in respect of mean age, marital state, and the number of sexual partners treated at the same time.

The importance of hygiene and of having an adequate diet was stressed and each patient was told that the partner(s) must be given treatment simultaneously.

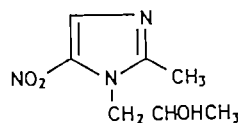
Diagnostic and control samples were taken as previously described (Siboulet *et al.*, 1977). The samples were examined microscopically—phase contrast and May-Grunwald-Giemsa stain—and cultured on Roiron's isolation medium (Durel *et al.*, 1961), before treatment and 48 hours and 15 days after.



Metronidazole



Tinidazole



Secnidazole*

* 1-(2'-hydroxypropyl)-2-methyl-5-nitroimidazole

Address for reprints: D. Videau, Institut de Biopharmacie, Rhône-Poulenc Santé, Paris

Received for publication 31 October 1977

The following biochemical investigations were undertaken to monitor possible adverse effects: blood count, blood urea, prothrombin index, and transaminases.

In vitro estimations of the minimal inhibitory concentration (MIC) and the minimal trichomonacidal concentration (MTC) were made by the method of Wallin and Forsgren (1974), modified by substituting Dubost medium (Cosar *et al.*, 1962) for the Diamond medium (Diamond, 1957), as the latter is too rich and allows too great a growth rate for the sensitivity test. Details of the techniques are given elsewhere (Siboulet *et al.*, 1977). The MIC and MTC estimations were made on 11 strains (one reference standard, maintained in the laboratory for 20 years, and 10 strains freshly isolated from humans) for each of the three drugs—metronidazole, secnidazole, and tinidazole.

In addition we studied serum concentrations for the three products both physicochemically (Populaire *et al.*, 1977, personal communication) and microbiologically (Cosar *et al.*, 1962; Videau, 1972) and obtained the same results. This was carried out by taking blood samples 1, 2, 3, 6, 9, 12, 24, 48, and 72 hours after a single oral dose (2 g) of each drug had been administered to 10 volunteers* (five men and five women). With the assay procedure the standard deviation was 7% and half-lives were calculated according to the methods of Riegelman *et al.* (1968) and Ritschel (1970).

Results

The single 2 g dose was curative at 48 hours in 136 (97.1%) of the 140 patients treated (Table 1). Four patients were cured by further doses—one more dose in three cases and two extra doses in the other. Long-term results (15 days) showed that six of the 136 patients who had been cured after a single dose either had a relapse or were reinfected. With the exception of nausea in 4%, tolerance to secnidazole was excellent. No adverse effects were detected in the control tests.

Table 1 *Distribution of patients treated and clinical results 48 hours after treatment*

Dose (500 mg tablets)	No. of patients	Results	
		Cured	Not cured
Total dose 2 g	140 (56 men 84 women)	136	4
Total dose 2.5 g	110 (53 men 57 women)	105	5

*Dr P. Pasquier, Hôpital de la Croix, St Simon, Paris

Short-term (48 hours) cure was obtained in 105 (95.4%) of the 110 patients given 2.5 g secnidazole. This resembled the cure rate obtained with the lower dose.

The MIC and MTC values for the three products, determined simultaneously in one study, are given in Table 2. The mean values were obtained from duplicate measurements of MIC and MTC values for each drug from 11 clinical strains of trichomonas and were determined for the confidence limits.

The half-life values calculated from the equations for the serum clearance curves, obtained from the mean serum concentrations measured physicochemically, are given in Table 3.

Lastly, the serum drug-clearance curves, obtained after oral administration of 2 g of secnidazole or tinidazole (mean of 10 volunteers) are given with standard error of the mean in the Figure, as well as the curve for metronidazole, for the same 2 g dose, taken from the literature (Wood and Monro, 1975).

Table 2 *Comparison of minimal lethal and inhibitory concentrations ($\mu\text{g/ml}$)*

Product	$P=0.05$	
	MIC	MTC
Metronidazole	0.60 (0.48–0.71)	0.42 (0.31–0.58)
Secnidazole	0.70 (0.53–0.86)	0.63 (0.44–0.81)
Tinidazole	1.125 (0.88–1.36)	0.75 (0.49–1.00)

Student's *t* test applied to the mean-degrees of freedom 15

Table 3 *Comparison of the pharmacokinetic parameters after a single 2 g dose*

Product (2 g)	Elimination serum half-life (hours)		
	Men	Women	Mean
Metronidazole	—	—	7.3
Secnidazole	20.0	14.0	17.0
Tinidazole	13.0	12.0	12.5

Table 4 *Results of Student's *t* test applied to the comparison of the MIC of two products (degrees of freedom 30)*

Drug	Tinidazole	Secnidazole
Metronidazole	$t=4.29 > 3.65$ $P < 0.001$	$t=1.07 > 2.04$
Tinidazole	—	$t=3.14 > 2.75$ $P < 0.01$

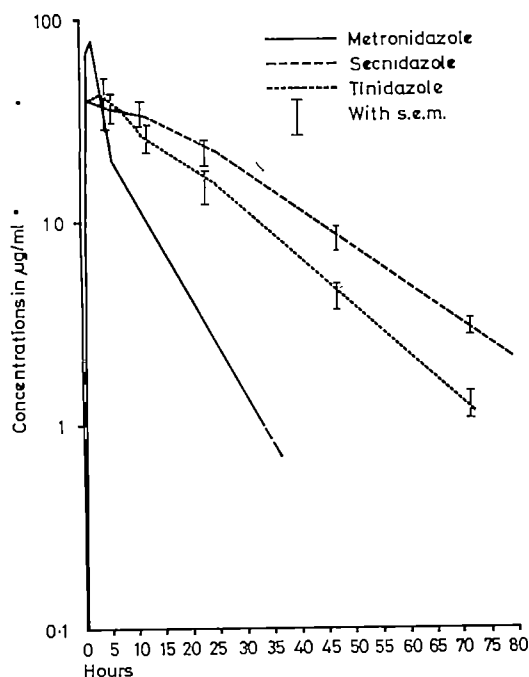


Figure Drug values in the serum after oral administration of 2 g of secnidazole or tinidazole showing mean from 10 volunteers and standard error of the mean. Curve for metronidazole (2 g dose) from Wood and Monro, 1975.

Discussion

With a single 2 g dose, 97% of the patients were cured, while the 3% failures responded to further doses of the drug (Table 1). There were 6-7% long-term relapses (on day 15) which may be related not only to the menstrual cycle and the probable presence of persistent forms of trichomonads but also to reinfection.

The values for metronidazole (90 to 92%) and tinidazole (96%) given by Wallin and Forsgren (1974) and 94% given by Swarz (1974) are comparable with those for secnidazole and the failures undoubtedly occur for the same reasons.

On the other hand, we believe that the different cure rates (which vary between 90 and 100%) reported in the literature for metronidazole-related compounds (for example, tinidazole and ornidazole) may be caused by differences in the patients and not by any therapeutic variations. Knowledge of the pharmacokinetic, trichomonostatic, and trichomonocidal properties of a drug could well be as useful as the cure rates obtained in clinical trials for assessing the efficiency of these drugs.

Statistical analysis by Student's *t* test of the MIC values (Tables 2 and 4) showed that there was no significant difference between the metronidazole and secnidazole MIC values but that there was a significant difference between these drugs and tinidazole, the latter being only approximately half as active. These results are in agreement with the work of Korner and Jensen (1976).

The study of the MTC showed that secnidazole continued to act on the parasite after being sub-cultured in a fresh medium and that the mean value of the MTC was always lower than that of the MIC. Statistical analysis (Student's *t* test) showed no significant difference in the trichomonocidal effect of the three drugs, but the mean lethal values for each drug bore the same relationship to the MIC values.

We agree completely with Wood and Monro (1975) that to ensure efficacy of a drug after a single dose the pharmacokinetics of the molecule, its half-life, and peak serum level must be understood. For secnidazole and tinidazole, the peak serum values, observed approximately three hours after administration of the dose, reached 40 and 46 µg/ml respectively and did not differ significantly. Nevertheless at between six and 72 hours, the level of secnidazole remained higher than that of tinidazole, and the Student's *t* test showed that the difference in the half-lives of the two products was highly significant ($P=0.05$).

Conclusion

Since the introduction of metronidazole in the treatment of urogenital trichomoniasis, other drugs have also been found to be effective for this disease, but they had different pharmacokinetic properties. Our studies demonstrated the efficacy of secnidazole (*in vitro* activity on *Trichomonas vaginalis* and its half-life in human serum) and showed that it is useful for single dose treatment of trichomoniasis.

The authors wish to thank May & Baker Ltd for help in the translation of this paper.

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Metronidazole and tinidazole in a single large dose for treating urogenital infections with *Trichomonas vaginalis* in men

NOBUO KAWAMURA

From the Department of Urology, Tokai University School of Medicine, Kanagawa, Japan

SUMMARY Seventy-three men with urogenital trichomonal infection were treated with a single daily dose of 1 g tinidazole or 1.5 g metronidazole. Both treatments gave satisfactory results. Wives who were also infected with *Trichomonas vaginalis*, were less likely to have a recurrence of the infection after their husbands had been given either drug.

Introduction

It has been pointed out by many investigators that more men would be found to have urogenital infection with *Trichomonas vaginalis* than actually reported if thorough examinations were performed (Strain, 1945; Bedoya *et al.*, 1958a, b; Schuppis, 1958; Catterall and Nicol, 1960; Ohmura, 1960; Hoffman *et al.*, 1961; Catterall, 1965; Kimura, 1965; Perl *et al.*, 1965; Schmör, 1974; Weidenbach and Leix, 1974). *T. vaginalis* is found in a little over 2% of normal male urine samples and in 8 to 11% of urine samples after prostatic massage. This detection rate rises to 10 to 13% when the spouses are infected with *T. vaginalis* (Nittono, 1959; Kawamura, 1969).

Male patients with trichomonal urogenital infections are usually asymptomatic or present only mild symptoms of urethritis or prostatitis (Freed, 1948; Keutel, 1959; Yamamoto, 1963; Wallin, 1974). Asymptomatic patients should be treated to prevent spread of pathogenic protozoa, particularly if the spouse suffers from trichomoniasis. In such cases it is evident (Sylvestre *et al.*, 1960; Mizuno, 1963) that both partners have to be treated simultaneously to prevent possible recurrence of the wife's infection.

Trichomonal infections respond to drugs much more effectively in men than in women (Kawamura, 1969, 1973a, b). Oral trichomonocides may cause gastrointestinal disturbances and it is difficult to persuade men to take oral treatment for 7 to 10 days; this is particularly so if there are no symptoms or they are mild, or if the infection is

present only in the wife. These factors may result in inadequate treatment.

The American Medical Association, Drug Evaluation, warns that metronidazole has not been completely cleared from carcinogenicity and its use should be limited to a maximum total dosage of 5.25 g. Prolonged use should therefore be avoided. Hitherto we have treated men with urogenital trichomonal infections with a 7 to 10-day oral course of nitrofurantoin, acetylfuratrizine, trichomycin, or metronidazole. However because of these problems, we compared a single oral dose of either metronidazole or tinidazole with the normal 7 to 10-day course.

Literature published in the USA and Europe shows that for single doses either 750 mg tinidazole or 2 g metronidazole is generally used (Csonka, 1971; Campbell, 1972; Morton, 1972; Ozaki, 1972; Woodcock, 1972; Dellenbach and Muller, 1974; Forsgren and Wallin, 1974; Wallin and Forsgren, 1974; Hayward and Roy, 1976). In view of the average body weight of the Japanese, however, either 1 g tinidazole or 1.5 g metronidazole was given.

Materials and method

Male outpatients from the departments of urology in Tokai University Hospital, Keio University Hospital, Nerima General Hospital, Saiseikai Kanagawa Prefecture Hospital, and Kawasaki City Hospital were selected during 1974-76. They had presented with symptoms of urethritis or prostatitis, were incidentally found to be infected with *T. vaginalis*, or had wives with trichomoniasis.

Address for reprints: N. Kawamura, Department of Urology, Tokai University School of Medicine, Kanagawa, Japan

Received for publication 23 June 1977

The patients who were selected in 1974 and at the beginning of 1975 were treated with tinidazole. After June 1975, patients were treated with metronidazole.

Examinations were carried out on morning urine, the first glass of Thompson's two-glass test, mid-stream urine, prostatic fluid, urethral secretion, and urine after prostatic massage. If the patient was married, the wife was investigated in the gynaecological or the urological department.

Cultures for *T. vaginalis* were done using mainly the Asami culture medium (Asami, 1952; Kawamura, 1969), and also the commercial Vaginal Trichomonas-Candida culture medium (product of Nissui Seiyaku Co. Ltd). In each case, urine sediment or secretion smear was examined microscopically. Some cases were detected only by microscopical examination.

T. vaginalis detection rates vary considerably according to the medium employed. Although the relevant data are not presented here, we believe the Asami culture medium is most suitable for the examination of trichomonal infection in men (Iijima, 1959; Nakabayashi, 1959; Matsuda, 1969, unpublished; Kawamura, 1976, unpublished). This is because trichomoniasis in men is characterised by a relatively small number of protozoa and this medium is capable of culturing even a single protozoon.

Altogether 73 patients were found to have trichomoniasis (Table 1). 1 g tinidazole or 1 g metronidazole was administered as a single oral dose.

Table 1 Total and *T. vaginalis* positive findings (73 patients)

	Total specimens	<i>T. vaginalis</i> positive specimens
Morning urine	12	3
First-glass urine	60	46
Midstream urine	58	23
Prostatic fluid	3	2
Urethral secretion	4	1
Urine after prostatic massage	46	32

As more than one specimen was tested for each patient and more than one specimen in many patients was found to be *T. vaginalis* positive the two columns do not add up to 73.

Drug evaluation was made by culturing between 7 and 14 days after treatment the same specimens that had been found to contain trichomonads before treatment. If *T. vaginalis* was not detected, the cases were judged as cured. In cases where the wives had been infected with trichomoniasis the husbands were asked to undergo investigation on two occasions during the 14-30-day period and a cure was judged if trichomonads were absent in all the specimens.

Results

The results are summarised in Table 2. All 73 men with trichomonal infection (tinidazole-treated group 39; metronidazole-treated group 34) were cured and there were no relapses.

Table 2 Results of treatment in 73 patients with *T. vaginalis*

Treatment	No. treated	No. cured
Tinidazole	39	39
Metronidazole	34	34

Six cases in the tinidazole-treated group and four cases of the metronidazole-treated group complained of gastrointestinal disturbances. These disappeared naturally within a few days. No marked difference in severity of these side effects was observed between the two drugs and no serious side effects were seen.

Nine patients in the metronidazole group had liver function tests and blood urea nitrogen was measured before and 7 to 14 days after treatment and a sperm count was taken. No abnormal values were found in any of the biochemical tests. In three cases the sperm count was reduced by more than 10 million and in a fourth case by 30 million.

Discussion

The dosage and method of administration of tinidazole and metronidazole can be considered adequate, judging by the results obtained in this study. No particular difference in efficacy was seen between the two drugs.

In view of the minor side effects, the dosage could safely be increased. The results of Hoffman *et al.* (1961) and Kawamura (1973a) suggest that the dosage might even be reduced with equally effective results. Clinical trials are under way to assess this point. Minimum inhibitory concentrations of these two drugs for *T. vaginalis* were determined (Kawamura, 1973b, 1974) using MSF medium; these were 0.6 to 1.2 mg/ml for tinidazole and 0.8 to 1.2 mg/ml for metronidazole. Bearing this in mind, as well as the blood levels for these two drugs (Ozaki, 1975; Wood and Monro, 1975; Seiga, 1976, unpublished), administration of metronidazole in a single dose of 0.75-1 g should be sufficient.

The results presented here are better than those reported by Morton (1972) and are in approximate agreement with those of Rodin *et al.* (1960) and Schapira (1965). The data are also compatible with the report of Perl (Perl *et al.*, 1965) that 98.2% of patients treated with 0.75 g metronidazole daily for 10 days were cured.

Trichomonal infections of the female genital tract may recur (Jones, 1972), but it is known that the cure rate improves if the husband is treated simultaneously (Schuppius, 1958; Perl and Ragazzoni, 1963; Campbell, 1972; Woodcock, 1972). As trichomonal infection in men can easily be controlled, as demonstrated in this study, treatment with a single dose large enough to be effective is advocated.

Neither tinidazole nor metronidazole is a safe drug. It is possible that as nitro-compounds they may have an adverse effect on spermatogenic function and they are not recommended for heavy drinkers or for prolonged medication. Treatment with a single, large dose eliminates these disadvantages.

The sample size of the present study (73 patients with urogenital trichomoniasis) is larger than in similar studies, and can perhaps serve as a useful reference on the clinical application of this treatment in the future.

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Abnormal forms of *Trichomonas vaginalis*

J. JOHN AND S. SQUIRES

From the Special Clinic, Derby Royal Infirmary and the Research Laboratories,
May & Baker Ltd, Dagenham, Essex

SUMMARY Abnormal forms of *Trichomonas vaginalis* have been demonstrated by both conventional and scanning electron microscopy after inoculation of media with clinical material from cases of trichomonal vaginitis. Twenty-six cases of vaginitis have been studied; 10 of them showed the abnormal forms of trichomonads after growth in a modification of the medium described by Bushby and Copp (1955), while 16 showed only normal forms.

Introduction

Abnormal forms of *Trichomonas vaginalis*, with variation in size and shape, have previously been reported. Marchand (1894) described a large parasite with three tufts of flagella and four nuclei, while other workers have also reported variations, particularly in the size of the parasite (Powell, 1936; Trussell, 1947; Winston, 1974). Some of these findings suggest that the shape varies according to the composition of the growth medium.

Recently, while investigating the effect of various additions to our standard culture medium (Squires and McFadzean, 1962), in order to increase its growth potential, we observed parasites with an abnormal morphology after subculture of the vaginal secretion. These abnormal trichomonads are described in this paper.

Materials and methods

Twenty-six female patients were studied. A diagnosis of trichomonal vaginitis was made after fresh vaginal discharge had been examined by darkground microscopy. Once a positive diagnosis was made, a sample of the vaginal discharge was inoculated into 20 ml of the medium described by Bushby and Copp (1955), modified by Squires and McFadzean (1962). The vaginal discharge was obtained by using a disposable vaginal pipette. The inoculated medium was placed in the incubator without delay and incubated at a temperature of 35-37°C for 24 hours. At this time, growth was visible in all 26 cultures as a whitish deposit at the bottom of the culture tubes.

Incubation was continued for 72 hours, at which time the growth was examined by darkground microscopy and the appearance and behaviour of the parasites were recorded. The growth was re-inoculated into fresh medium and incubated as before, again the growth was examined on the third day. This re-inoculation process was repeated 10 times for a total observation period of one month.

The growth from the cultures of one patient, which showed a large number of bizarre forms, was also studied under a scanning electron microscope. The specimens were prepared by fixing two to three drops of the centrifuged (at 500 rev/min for five minutes) culture deposit in 10 ml of ice-cooled glutaraldehyde for one hour; this was then again gently centrifuged and the deposit washed in cacodylate solution. After being centrifuged again as above, the deposit was passed through a series of acetone washes (25%, 50%, 75%, and 100%), and allowed to remain in each concentration for 30 minutes. A drop of the suspension from the final acetone wash was then placed on a coverslip under a drop of 100% acetone. The coverslip was then cemented with a current-conducting glue and coated with gold before being examined by the scanning electron microscope.

Results

Of the 26 cases studied, 10 gave cultures of abnormal *T. vaginalis* and 16 showed only normal forms. None of the 26 patients had shown abnormal forms in the fresh vaginal discharge examined before culture. The abnormal forms started to appear in the first or second passage, and were then seen persistently in the subsequent subcultures, but in

Address for reprints: S. Squires, Research Laboratories, May & Baker Ltd, Dagenham, Essex
Received for publication 30 August 1977

varying numbers and always intermixed with normal forms. The abnormal parasites appeared under the light microscope to be large and irregular in shape, with multiple nuclei, multiple flagellae, and amoeboid movement (Fig. 1). Large, rounded, non-motile or sluggishly motile forms with a hyaline appearance were also seen, but these were obviously dead (encysted) or dying organisms. Under the scanning electron microscope the following morphology was noted: abnormal forms were larger than organisms of normally accepted size and had irregular surfaces with spiky projections (Fig. 2), or

had multiple stumps and fragmented undulating membrane (Fig. 3). It is possible that some of these stumps were flagellae which had been damaged in the course of preparing the microscopical material; or it is more likely that they were the pseudopodia by which the parasite ingests bacteria or other particulate matter. Figure 4 shows a normal *T. vaginalis* as seen under the scanning electron microscope.

Cultures showed a very high proportion of abnormal forms in the vaginal discharge of a 16-year-old girl, so these were studied under the scanning micro-

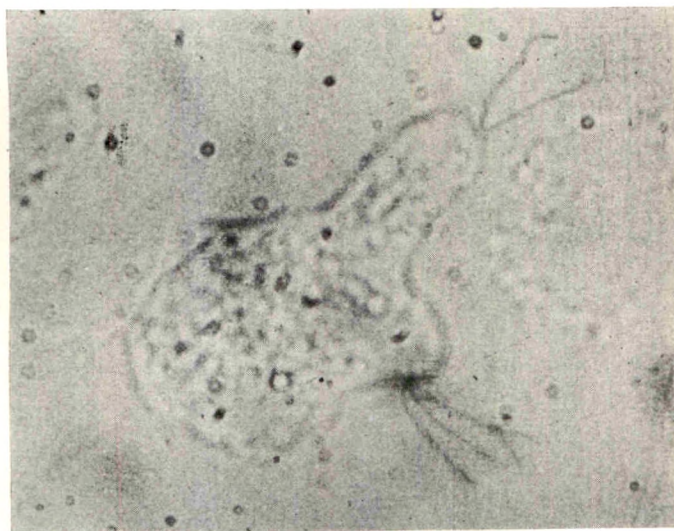


Fig. 1 Abnormal *T. vaginalis* showing multiple nuclei and flagellae. Normal microscopy.

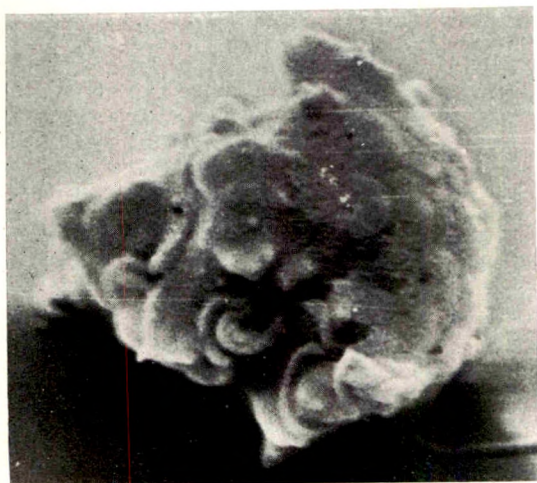


Fig. 2 Abnormal *T. vaginalis* showing spiky projections. Scanning electron microscope.

scope. *T. vaginalis* infection had been diagnosed on seven occasions during a 30-month period. She had had a gonococcal infection on eight occasions and other sexually transmitted infections separately or in association with *T. vaginalis*.

Of the 10 patients who showed abnormal forms of the parasite, three were pregnant at the time of examination and already had children, another three were not pregnant but had previously had children, and four of them were multipara. The group which showed only normal forms were all nulliparous.

There were patients with and without abnormal trichomonads who had gonorrhoea, candidosis, genital herpes, and warts in association with trichomoniasis. However, it is an interesting observation that the history of the group with the abnormal forms shows they had sexual intercourse more often and with more partners than those with only normal forms.

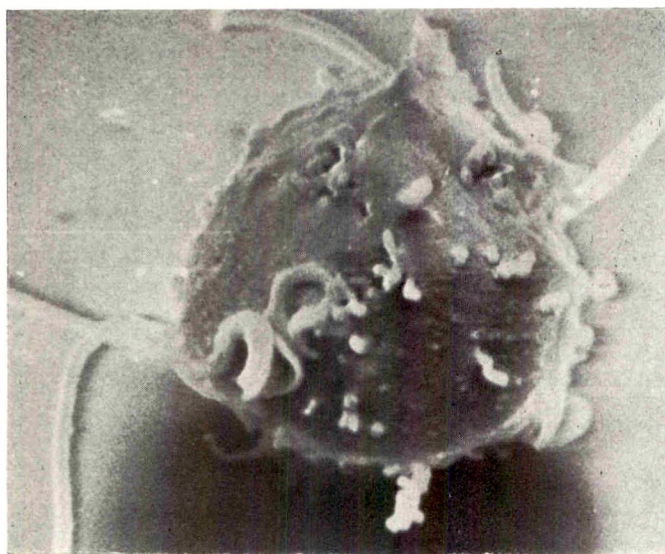


Fig. 3 *Abnormal T. vaginalis with multiple stumps and fragmented undulating membrane. Scanning electron microscope.*

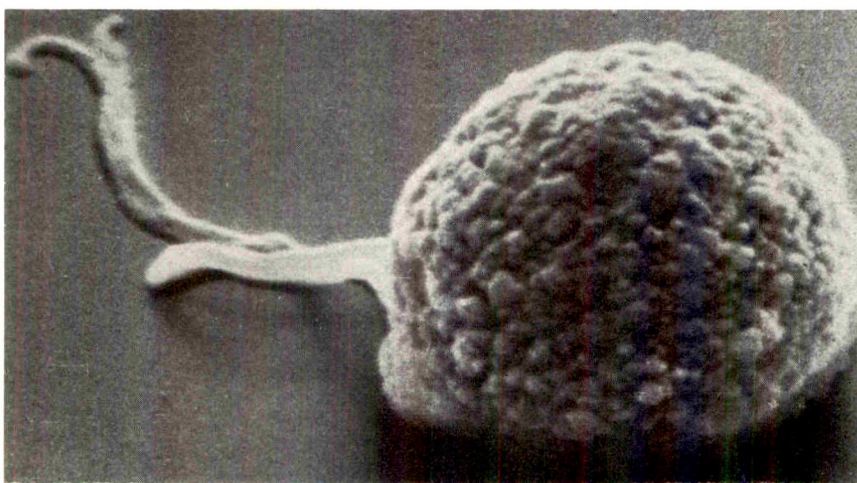


Fig. 4 *Normal T. vaginalis. Scanning electron microscope.*

The abnormal forms were tested for their sensitivity to metronidazole and were found to be as sensitive as the normal forms.

Discussion

Abnormal forms of *T. vaginalis*, with variation in size and shape have previously been described. Wenrich (1930) reported trichomonads varying in size between 7 and 23 μm , Whittington (1957) observed some as large as 100 μm , and Winston (1974) reported on giant forms measuring between 30 and 50 μm in diameter and appearing in liquid

medium 12 days after incubation. Multinucleated and multiflagellated forms were also observed. There are also reports of variation in shape depending on the environment, some authors claiming that the shape of the organism varies according to the composition of the growth medium (Künstler, 1883). It has also been suggested that the morphology of the parasite varies according to the external environment, but the smaller the organism the more regular is the shape. Honigberg and King (1964), using phase contrast microscopy, showed ellipsoidal, ovoid, and spherical forms; they also suggested that pseudopodium-like protrusions could be seen.

In addition to the process of reproduction, usually accepted as being one of binary fission (Donné, 1836), Marchand (1894) reported multiple fission forms and described a large trichomonad with three tufts of flagellar apparatus and four nuclei. These observations have also been supported by other workers. Powell (1936) described an organism with two nuclei and four sets of flagella, Trussell (1947) noticed in cultures of trichomonads some with eight nuclei, while Winston (1974) reported giant forms which contained a dozen or more nuclei with multiple blepharoplasts each giving rise to three or four flagellae. The abnormal forms seen in our observations showed a larger size than normal, irregular in shape, and with multiple nuclei. Furthermore, they were seen only after subculture in liquid medium. Winston (1974) was of the opinion that, in a favourable environment, the organisms reproduce rapidly, assuming a small size, and these cause the inflammation and symptoms of the disease, whereas when the environment is unfavourable the parasite reproduces slowly, becomes larger, and lives as a saprophyte. Kupferberg (1940) showed that at an unfavourable pH, the rate of multiplication decreased, and large trichomonads appeared in the cultures.

The significance of these abnormal forms is still to be assessed. Are they in any way related to the long-standing use of chemotherapeutic agents? (some were described before the use of drugs); or are they forms capable of multiple fission in response to the adverse environment in which they were situated—a prolonged disease state? We feel that the relapse of patients from whom these abnormal forms are isolated should now be investigated, and that the influence of the constituents of the culture medium on their development should also be studied.

We should like to thank Dr G. W. Csonka and Dr W. H. Donald for their advice and encouragement, and to the staff of the Wolfson Institute of Interfacial Technology, particularly Mr L. Green, for their expert help with the scanning electron microscopy.

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Strain related infectivity of *Neisseria gonorrhoeae* for the guinea-pig subcutaneous chamber and the variability of the immune resistance in different breeds of guinea-pig

P. NOVOTNY, E. S. BROUGHTON, K. COWNLEY, M. HUGHES, AND W. H. TURNER

From the Department of Bacteriology, Wellcome Research Laboratories, Beckenham, Kent

SUMMARY The ability of gonococci to infect the subcutaneous chamber in guinea-pigs is strictly strain related. This ability was usually present in prototrophic strains and auxotrophs requiring proline only which were obtained from patients with uncomplicated gonorrhoea, but it was invariably absent in *Neisseria gonorrhoeae* auxotypes requiring arginine, uracil, and hypoxanthine, or arginine only for growth. All the strains obtained from disseminated gonococcal infections (all dependent upon arginine, uracil, and hypoxanthine) were unable to infect the guinea-pig chamber. Hence, the high invasiveness of *N. gonorrhoeae* for man and its infectivity for guinea-pig chambers appear to be unrelated properties. Although guinea-pigs of the same origin (Dunkin-Hartley) were used throughout, the degree of immune resistance was found to differ between the lines supplied by various breeders—that is, after a standard immunisation schedule using whole cell gonococcal vaccines, the homologous immune resistance to challenge varied from weak or non-existent in some lines, to highly resistant in others.

Introduction

The guinea-pig subcutaneous chamber model as pioneered by Arko (1972, 1973), is gaining interest in gonococcal research. This is because the T₁-T₄ colonial forms of gonococci can infect the chambers more easily than types T₅-T₆ and are able to survive therein for longer periods of time, and because active (Arko, 1974) and passive (Arko, 1974; Scales and Kraus, 1974) immunisation can be used to protect against an infectious challenge. Using this technique, it has been possible to (i) demonstrate several immunotypes (Arko *et al.*, 1976a), (ii) show that pillar antibodies do not confer immunity to infection (Turner and Novotny, 1976), and that experimental cross-protection is related to bactericidal antibodies (Wong *et al.*, 1976). Of greater significance is the finding that the increased immune resistance observed after the artificial infection or vaccination of chimpanzees broadly corresponds

with results obtained in experiments with guinea pigs (Arko and Wong, 1977), and it is obvious that guinea-pigs are attractive for *in vivo* studies of gonococcal physiology (Veale *et al.*, 1975; Arko *et al.*, 1976b; Penn *et al.*, 1976, 1977a,b; Novotny *et al.*, 1977).

It is not known whether the guinea-pig model has a direct relationship with the immune state in man. There is a basic difference between the infectious process in man and in guinea-pigs. In the latter infectious units are not formed (Novotny *et al.*, 1977), and immunologically the guinea-pig recognises gonococci in a different way (Miler *et al.*, 1977). Therefore, only exhaustive comparative studies would be able to show how knowledge obtained from a guinea-pig model would apply to the physiology and immunity of the gonococcal infection in man.

The guinea-pig model has been used extensively in this laboratory, and two qualities have become apparent. The first is related to the genotype of the gonococcus; certain freshly isolated gonococcal strains are non-infective for the chamber. The

Address for reprints: P. Novotny, Department of Bacteriology, Wellcome Research Laboratories, Beckenham, Kent BR3 3BS

Received for publication 26 September 1977

second is related to the genotype of the guinea-pigs—two distinct lines were found, one of which, when immunised, showed a high immune resistance to infection, while the other was barely protected.

Methods

CULTURE MEDIUM

GC medium plates were prepared from GC medium base (Difco) enriched with glucose 22 $\mu\text{mol/ml}$, L-glutamine 0.68 $\mu\text{mol/ml}$, $\text{Fe}(\text{NO}_3)_3$ 0.1 $\mu\text{mol/ml}$, and cocarboxylase 4.35×10^{-4} $\mu\text{mol/ml}$, all added as filter-sterilised solutions. The cultures were incubated at 37°C in jars containing 5 to 10% CO_2 .

GNOCOCCAL STRAINS

The same strains were used as in previous studies (Novotny *et al.*, 1975a,b; Novotny and Turner, 1975; Turner and Novotny, 1976; Novotny *et al.*, 1977), all being obtained from the urethra and/or cervix of patients with gonorrhoea. The growth from primary cultures on GC medium or GC medium with VCN inhibitor (Baltimore Biological Laboratories) was washed off using a protective medium (Turner and Novotny, 1976) and stored in the gas phase of a liquid nitrogen refrigerator (LD40, Union Carbide Limited) as the primary master culture (PMC). When the primary cultures were contaminated, the T_1 – T_4 colonies of gonococci were subcultured once on GC plates and then washed off and stored as the primary subculture (PS). To obtain seeds from frozen suspensions, the technique described by Bullen (1975) was used; using the serrated sampling tool a small amount of the suspension could be scraped from the frozen block, thus avoiding any thawing of the master suspensions. The cultures were identified by colonial morphology, oxidase, and fermentation tests (Kellogg and Turner, 1973). The cultures mainly consisted of T_1 or T_2 colonial forms. Where possible, subculturing of T_3 and T_4 forms was avoided.

AUXOTYPE DETERMINATIONS

These were made using the NEDA media and the technique described by Catlin (1973) and Carifo and Catlin (1973). As after the recommended purification procedure, Difco Noble Agar was still inhibitory to some strains when used in the NEDA medium, 0.1% mass/vol. of soluble starch (Analar, BDH) was incorporated into the agar base (Professor Catlin, personal recommendation). Although impurities in the agar are usually blamed for the inhibitory effect, we found that the collection of glass bi-distilled water in a polyethylene receiver, rendered the water unsuitable and that the inhibitory effect thus caused could not be removed by adding

starch. Therefore, contact with any sort of plastic during medium preparation was avoided. Individual batches of auxotyping media were checked by the use of the reference strains used by Carifo and Catlin (1973)—namely, strains ODH2915, MHD340, MHD361, SS7764/45, MCH3317/70, and MHD446—all of which were obtained from the National Collection of Type Cultures, Colindale, London NW9 5HT.

GUINEA-PIGS

Dunkin-Hartley outbred animals, 500–800 g, were supplied by Charles River, UK Limited, Margate, Kent; Porcellus Animal Breeding Limited, Heathfield, Sussex; Olac, Bicester, Oxon; Redfern Animal Breeders Limited, Brenchley, Kent.

IMPLANTATION OF THE CHAMBERS

The technique used was similar to that described previously (Turner and Novotny, 1976) with the following modifications. Twenty-four hours before surgery, the abdominal area of the guinea-pigs was clipped, and the guinea-pigs were supplied with drinking water containing 200 mg/litre of Terramycin soluble powder (Pfizer Limited, Sandwich); this represented about 20–50 μg of oxytetracycline per gram of animal weight a day. The supply of antibiotic was discontinued two to three days after chamber implantation. Ten minutes before surgery, the animals were injected intraperitoneally with 0.8 ml/kg body weight of 1% sodium thiopentone (Intraval Sodium, May and Baker, Dagenham). In some of the guinea-pigs a short inhalation of ether anaesthetic vapour was necessary to deepen the narcosis. The skin was thoroughly disinfected with 5% tincture of iodine and the narcotised animals were fixed within a sterile Medioplast sterilising bag (No. A-10, supplied by Henly's Medical Supplies, London N8) in which, using a sterile pair of scissors, a window of about 5 × 3 cm had been cut in the transparent side of the bag, just above the operating area. This produced a 'clean surgical site' which was shielded from the rest of the guinea-pig. Through this window, two paracentral ventral incisions, 2 cm long, were made and then, using artery forceps, two subcutaneous pockets were prepared, into each of which two chambers were implanted. The chambers were positioned wide apart under the skin, which was then sutured using surgical braided nylon (Finlayson's metric no. 5) and finally dressed using Nobecutane spray (BDH). In this way each guinea-pig received four isolated chambers. The chambers were prepared from 2 ml polypropylene heat sealable ampoules (no. 508S, Sterilin Limited, Teddington, Middlesex), which had had the narrow



necks cut off and the sides perforated with 12 holes (approximately 2×5 mm) using a grinding wheel. The total area of the holes (about one square centimetre) represented approximately one-tenth to one-twelfth of the total surface area of the ampoule. The chambers were sterilised in an autoclave (15 minutes at 120°C). The guinea-pigs were operated on in groups of five—usually 10 groups a day. Using this aseptic surgical technique, none of the implanted chambers became infected. If these precautions were neglected, this usually led to a high rate of chamber infection followed by subsequent rejection by some of the guinea-pigs.

INFECTION OF THE CHAMBERS

For this, cells harvested from the first subculture on GC plates were used. The plates were inoculated directly from the frozen PMC or PS and after 18–20 hours' incubation, colonies were resuspended in phosphate buffered saline (PBS) (0.123 mol/l NaCl , $0.01 \text{ mol/l Na}_2\text{HPO}_4$, $0.0032 \text{ mol/l KH}_2\text{PO}_4$; pH 7.2) so that the injected volume of 0.2 ml contained approximately 10^7 colony forming units (cfu). The densities were adjusted spectrophotometrically (Coleman Junior II, at 650 nm). Although the spectrophotometer readings had been calibrated for a number of strains, the actual number of cfu contained in the inoculum was found to be strain dependent and varied greatly (10^7 to 10^9). The actual number of cfu was therefore determined by plate counts (Novotny *et al.*, 1977) in each case. The gonococcal suspensions were injected within 30 minutes of preparation, during which time no decrease in viable count was observed.

In several cases, the guinea-pig chambers were injected directly with pus from human males. Pus from the urethra was aspirated using a 1 ml syringe and immediately emulsified with an equal volume of VCN inhibitor, which had been diluted 1:50 in PBS. The mixtures, usually 0.1 to 0.2 ml in volume, were injected into the chambers after being left for 30 minutes at room temperature. Three to five days later, samples of the chamber contents were inoculated on to GC plates. About one-fifth of these samples rendered pure cultures of gonococci which were stored frozen as above.

CHALLENGE SUSPENSIONS

These were prepared from the PMC or PS in the same way as the suspensions for the chamber infection, or from the culture obtained from the infected chambers. Three challenge schedules were used:

1. PMC (or PS) $\rightarrow$ GC plate $\rightarrow$ challenge, or
2. PMC (or PS) $\rightarrow$ GC plate $\rightarrow$ GP chamber $\rightarrow$ GC plate $\rightarrow$ challenge, or

3. PMC (or PS) $\rightarrow$ GC plate $\rightarrow$ GP chamber $\rightarrow$ GC plate $\rightarrow$ frozen suspension $\rightarrow$ GC plate $\rightarrow$ challenge. The suspensions were diluted and standardised as above. The challenge suspension was regarded as adequate if it was possible to establish gonococcal infection in all of the inoculated chambers of the non-immune guinea-pigs, the infection having to persist for at least 14 days.

TEST FOR CHAMBER INFECTION

A small sample of the chamber contents (about 0.1 ml) was aspirated using a 2 ml syringe and needle through the iodinated skin covering the chamber. One drop of this liquid (about 50 μl) was spread over a GC plate. Since the maximum volume of chamber liquid obtainable by this method was about 0.5 ml, this count volume represented about one-tenth of the chamber liquid. Inoculated plates were incubated for 18 to 24 hours, and the chamber was regarded as being infected when gonococci could be identified in the cultures. Growth varied from 1–2 colonies to confluent gonococcal growth. Each chamber was sampled several times—usually on the third and seventh day after gonococcal inoculation, and later at appropriate intervals. The chamber was regarded as being non-infected when no gonococcal growth was obtained on three successive occasions.

AGGLUTINATION TEST

The agglutination antigen was prepared as recommended by Arko (Arko, 1974, and personal communication). The growth from the first culture of the infected guinea-pig chamber liquid on GC medium was washed off with PBS, 0.5% v/v of 37% formalin was added, and the cell suspension was incubated for four hours at room temperature. The formalised cells were centrifuged (3000 *g* for 10 minutes), and resuspended in PBS to a density of approximately 1 mg dry weight of cells per ml; the antigen was used on the day of preparation. Under these conditions, most strains gave a smooth suspension which did not autoagglutinate, but which deteriorated if kept at 4°C overnight.

Serial twofold dilutions of sera were made in plastic trays (U-shaped wells, IS-MRC-96, Linbro) using Microtiter loops. An equal volume (0.05 ml) of the antigen was added to each well, the trays were sealed with self-adhesive membranes, and then shaken to mix the reaction mixtures. After four hours' incubation at 37°C the plates were read using a Nikon plate microscope and oblique illumination. Positive results were compared with the control well (with serum omitted) and when autoagglutination occurred the test was repeated the next day with fresh antigen. The gonococcal sera were tested only against their homologous antigen.

VACCINES

Gonococci from the frozen PMC or PS after one or two subcultures on GC plates were suspended in PBS containing 0.5% v/v 37% neutral formalin solution, incubated overnight at 4°C, sedimented by centrifugation (3000 g, for 10 minutes), and resuspended in PBS. One ml of the final vaccine contained the equivalent of 10^8 cfu, 1 mg of A1 (added as Alhydrogel, Superflos, Kobenhagen) and 1:10 000 Merthiolate. The Alhydrogel was added routinely not primarily as an adjuvant, but as a detoxifying agent, since without it some of the vaccines were dermonecrotic. The vaccines were standardised in the same way as the challenge culture preparations. The vaccines were used within one and four weeks of their preparation. After storage at 4°C for about a year, the vaccines showed no apparent loss of protective activity under the particular test conditions, although considerable lysis of the cells was seen to have occurred when checked by an optical microscope.

For vaccines prepared from gonococci grown in embryonated eggs (10 days, White Leghorn), a previously reported modification (Novotny *et al.*, 1977) of the method of Gibbs and Roberts (1975) was used. The seed eggs were inoculated with gonococci harvested from the first to third subculture on GC plates, and the culture from this passage in eggs was used to inoculate the production batch of eggs. After five hours' incubation at 37°C on an orbital shaker (Kuhner, Basel, Switzerland, type RC5SP) the allantoic fluid was harvested, centrifuged for 10 minutes at 800 g to remove the embryonal cells, and the supernatant respun at 6000 g to recover the gonococci. These were resuspended in PBS to a density of about 10^8 cfu per ml and formalised (0.5% v/v, 37% formalin) overnight at 4°C. The formalised cell suspension after centrifugal washing was standardised as above.

COMPLEMENT TITRE DETERMINATION

The method of Mayer was used (Kabat and Mayer, 1961).

Results

INFECTIVITY OF FRESH ISOLATES OF GONOCOCCI FOR SUBCUTANEOUS GUINEA-PIG CHAMBERS

In most of our experiments, the strains tested for their ability to infect guinea-pig chambers were used as a suspension of colonies from the first to third subculture on GC medium. This passage on artificial medium was not necessary, since in several cases it was possible, in the presence of VCN inhibitor, to infect the chamber directly with human pus.

Initially, it appeared that all of our strains were infective—that is, they were able to establish an infection of the chamber, which subsequently lasted for several weeks, from an injection of approximately 10^7 cfu from T₁–T₃ colonial forms. Later, the infection subsided spontaneously. Using fresh isolates the primary infection of the chambers did not usually last longer than two or three weeks. However, if the infected chamber contents were then transferred to a second guinea-pig chamber, or if the chamber liquid was cultured on GC plates and the colonies obtained by this single passage on artificial medium were used as a chamber inoculum, in most cases the infection of the chambers then lasted longer. With some strains—for example, Pat 1, StG 18, StG 4, StG 38—the infection lasted for six months or more (Turner and Novotny, 1976). It appears, therefore, that the survival period of gonococci in the guinea-pig chamber depends largely on selective conditions, passaged gonococci being eliminated from the chamber less readily than fresh isolates.

Eventually, we tried to test for passive protection in guinea-pigs using serum obtained from convalescents with disseminated gonococcal infection; experiments had shown that the serum, when administered intramuscularly, rapidly penetrated the chamber contents (Figure). However, we found

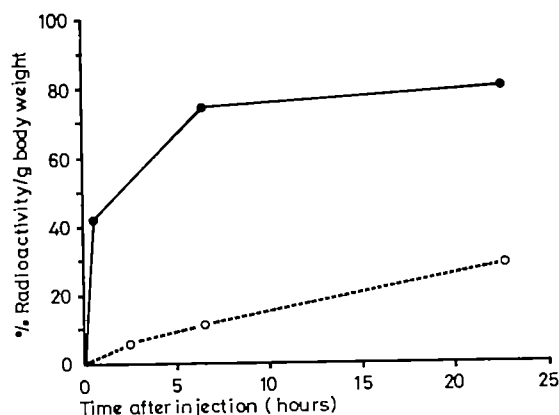


Figure Penetration of passively administered human antigonococcal IgG into subcutaneous chambers in guinea-pigs

Human antigonococcal IgG labelled with ^{125}I (Novotny *et al.*, 1977) was injected intramuscularly into seven guinea-pigs with non-infected chambers, samples were taken at appropriate intervals.

The results are expressed as the mean percentage of the total radioactivity injected per g of body weight.

● — — — ● Blood ○ — — — ○ Chamber liquid

that we were unable to infect the chambers using the strains obtained from these patients (Novotny *et al.*, 1977). All of these strains behaved as typical DGI (disseminated gonococcal infection) strains (Holmes *et al.*, 1976; Schoolnik *et al.*, 1976)—that is, they were highly sensitive to penicillin, resistant to the bactericidal effect of human sera, and they required arginine, uracil, and hypoxanthine for growth. Despite intensive efforts to infect the chambers with these strains, testing for passive protection using human sera proved impossible, since using animal immune sera this test was found to be strain specific (Scales and Kraus, 1974, and unpublished observations). In the course of further experiments, other strains, which although originating from cases of uncomplicated gonorrhoea, were also found non-infective for the chamber. When auxotypes of all of our strains had been determined (Table 1) it became apparent, that none of the strains requiring arginine, uracil, and hypoxanthine for growth—whether from generalised infection or not—was infective. Another strain (StG 41) requiring arginine only, was also non-infective. On the other hand, with three exceptions, prototrophic strains or strains requiring proline only, obtained from patients with uncomplicated gonorrhoea, were able to establish infection when injected into the chamber. Two isolates from corresponding consorts, StG 102 and 104 (Novotny *et al.*, 1975b), although auxotrophs requiring proline and arginine were infective. We suggest therefore, that the ability to infect the guinea-pig chamber is a specific characteristic of the wild strain, being absent in auxotrophic strains requiring arginine, uracil and hypoxanthine, but which, occasionally, may also be absent in some prototrophic strains. This inability to infect the chamber appears to be a stable feature, since isolates StG 90 and 174C, when subcultured did not gain this property even after 10 passages on GC medium.

INDUCED IMMUNITY FOR GONOCOCCAL CHAMBER INFECTION

After preliminary experiments, a test system was developed which enabled us to perform routine determinations of the active immunity of guinea-pig chambers against gonococcal infection. In this test the guinea-pigs were injected intramuscularly or subcutaneously with two 1 ml doses of a vaccine, containing an equivalent of about 10^8 cfu per ml of gonococci in an aluminium gel. The doses were injected at an interval of between two and three weeks. The chambers, implanted one week before immunisation, were challenged with a suspension of the homologous strain containing approximately 10^7 cfu. With most strains the size of the challenge did not appear to be critical, provided that the challenge dose fell between 10^5 and 10^8 cfu (Table 2). Higher challenges were usually preferred since, with some fresh isolates, the control chambers occasionally failed to be infected by low challenge doses. Under these conditions, vaccines prepared from most of the infective strains listed in Table 1 induced solid immunity—that is, all the chambers were protected against the homologous challenge. This protection was independent of the nature of the challenge culture whether being in the form of a fresh isolate or an isolate obtained after one or several passages through the guinea-pig chambers. Vaccines prepared from some strains (StG 38, Pat 1) conferred protection even at a dilution of one-fifth of the neat vaccine dose, but were not usually protective at a dilution of 1:25. On the other hand, some fresh isolates conferred only feeble protection (for instance the vaccine prepared from strain StG 4 protected only six out of 17 chambers), while vaccines prepared from strains StG 18 and StG 76 were not able to protect any chamber. However, the protective potency of the whole cell vaccine could be increased by changing the conditions under which the vaccine cells were cultured. For instance

Table 1 *Growth requirements and infectivity of N. gonorrhoeae strains for subcutaneous guinea-pig chambers*

Strain no.	Growth requirements*					Ability to infect guinea-pig chambers†
	Auxotype*	Proline	Arginine	Hypoxanthin	Uracil	
StG 2, 4, 6, 18, 19, 25, 38, 76, 79, 86, 87U, 93, 94, 95, Pat 1, A4385	1 or 2	— or +	—	—	—	+
StG 48, 90, 174C	1	—	—	—	—	—
StG 41	3	—	+	—	—	—
StG 102, 104	7	+	+	—	—	+
StG 127C, 127U, 128C, 128U, 176C, 176U (= DGI† strains)	14	—	+	+	+	—
StG 45, 77, 80C (DGI qualities not tested)	14	—	+	+	+	—

*Determined on NEDA medium according to Catlin (1973) and Carifo and Catlin (1973)

†The non-infective strains were tested on at least four chambers, most on 8 to 24 chambers and strains StG 90 and StG 174C on 65 and 84 chambers, respectively

‡Disseminated gonococcal infection

Table 2 *Gonococcal protective test in Charles River guinea-pigs after immunisation with GC-plate grown vaccines*

Vaccine strain	Homologous challenge dose (cfu)	Unvaccinated controls	Vaccinated with no. of doses*		
			2	3	7
StG 102	1.5 × 10 ⁸	2/2†	0/5	2/6	0/6
	3.0 × 10 ⁸	2/2	0/5	0/6	0/6
	6.0 × 10 ⁴	3/3	0/3	0/6	0/6
	1.2 × 10 ³	3/3	0/5	0/6	0/6
StG 183	9.0 × 10 ⁸	4/4	0/4	0/6	0/6
	1.8 × 10 ⁷	4/4	0/4	0/6	1/6
	3.6 × 10 ⁵	3/4	0/5	0/6	0/6
	7.2 × 10 ³	3/3	0/5	0/6	0/5

*The vaccine doses were applied intramuscularly at three-week intervals (when 2 or 3 doses were given) or one-week intervals in guinea-pigs receiving 7 doses. All guinea-pig chambers were challenged 10 days after the last vaccine injection.

†The ratio represents the number of positive infections per number of chambers challenged.

a vaccine prepared from strain StG 76 grown in the allantoic fluid of embryonated eggs, conferred protection after one vaccine dose (about half the chambers being protected) followed by solid immunity after two doses (six out of six chambers being protected). However, the same strain grown on GC plates did not confer protection even after two immunising doses of the vaccine, the cells having been subjected to a similar number of passages (Table 3). It is evident, therefore, that the production of the protective antigen(s) necessary for the development of an immune resistance in guinea-pig chambers may be largely dependent upon cultural conditions and that in some strains, this protective antigen may be present at a rather low level.

These tests were performed to investigate cross-protection between different strains. However, inconsistencies began to appear in our results, in that vaccines prepared from strains which had previously been found to be protective, failed to confer protection. It was originally supposed that cultural conditions were responsible. However, we later realised that these irreproducible results appeared when the Dunkin-Hartley guinea-pigs supplied by Charles River became temporarily unavailable, thus making it necessary to obtain

guinea-pigs from other breeders. This can be seen by analysing the results of 1416 chambers, 686 of which were implanted into Charles River guinea-pigs, 342 into Olac or Redfern guinea-pigs, 300 into Porcellus guinea-pigs, and 88 into guinea-pigs of unknown origin. In the Olac and Redfern guinea-pigs, no homologous protection was conferred in 93% of the tests. In guinea-pigs supplied by Porcellus, the immune resistance was variable, some of the batches being well protected, while other batches failed to show protection with vaccines which had previously been shown to be protective. Only guinea-pigs supplied by Charles River rendered reproducible results, a situation which was confirmed when these guinea-pigs once again became available. As can be seen from Table 2, the protection is independent of both the number of immunising doses (2, 3, or 7) and the size of the challenge. On the other hand, in Olac and Redfern guinea-pigs the immune resistance where present is critically dependent upon the challenge dose (Table 3). Only those chambers which received relatively small challenge doses (<10³ cfu) appeared protected, whereas with higher challenge doses infection was established in many of the chambers. This remained the case even when the immunising dose was increased tenfold.

Table 3 *Gonococcal protection test in Redfern and Charles River guinea-pigs after immunisation with two doses of vaccine (strain StG 76)*

Vaccine origin	Homologous challenge dose* (cfu)	Unvaccinated controls	Vaccinated	Guinea-pigs
Eggs	6.0 × 10 ⁷	5/5†	6/10	Redfern
	1.2 × 10 ⁸	4/4	6/9	
	2.4 × 10 ⁴	4/5	5/10	
	4.8 × 10 ³	5/5	1/7	
Eggs	2.0 × 10 ⁷	8/8	0/6	Charles River
GC-plate			3/3	

*The guinea-pig chambers were challenged eight days after the final vaccine injection.

†The ratio represents the number of positive infections per number of chambers challenged.

A comparison of the agglutination titres of the sera between Charles River and Olac guinea-pigs showed that there was no difference in these two breeds (Table 4). Although there were more non-responders among the Olac guinea-pigs, the general trend of these guinea-pigs to form high agglutination titres is apparent. However, despite this fact, the agglutination titre was found to be unrelated to the protective activity, as similarly reported by Arko *et al.* (1976b). Similarly, both groups of guinea-pigs showed high titres of antibodies reacting with free endotoxin particles and ring structures, both derived from the outer cell membrane, with particles derived from mesosome-like bodies and with pili, as determined by immune electron microscopy (Novotny and Turner, 1975; Novotny *et al.*, 1977). Titres of antipilar antibodies were especially high, often being detectable at dilutions of 1:10 000 or more (unpublished observations). Both groups of guinea-pigs were similar in their complement titre which was between 180–240 C'H₅₀ units per ml.

Discussion

Some gonococcal strains are fastidious in their growth requirements, and strains obtained from patients with generalised infection usually belong to this category. This has been described in Seattle (Knapp and Holmes, 1975; Holmes *et al.*, 1976; Schoolnik, 1976), and has led to the definition of a distinctive group of gonococci—the DGI strains—which are characterised by (1) their increased sensitivity to inhibitory factors in the culture media, (2) their low antibiotic resistance (especially to penicillin), (3) their resistance to the complement dependent bactericidal action of normal human sera, (4) their resistance or relative low sensitivity to the cidal effect of acute or convalescent sera, and, in most reported cases, (5) by their belonging to an auxotype requiring arginine, uracil, and hypoxanthine for growth. Although this relationship between the ability to produce the disseminated gonococcal infection and the markers mentioned is not absolute, there are a few exceptions. The determination of these properties appears to be

important, since most of the highly invasive strains, and, probably, strains responsible for asymptomatic infections (Holmes *et al.*, 1976), belong to this category. Hence an important laboratory criterion has been proposed for distinguishing this most virulent (invasive) and, from an epidemiological point of view, very important group of gonococci.

Although strains of gonococci which are non-infective for guinea-pig chambers (Arko and Wong, 1977) or for chimpanzees (Arko *et al.*, 1974) are known, our study has shown that all of our auxotypes which require arginine, uracil and hypoxanthine for growth, including the DGI strains and a strain requiring arginine only were unable to infect guinea-pig chambers, whereas most prototrophic strains obtained from patients with uncomplicated gonorrhoea were infective. This property, therefore, could be added as another marker to differentiate between gonococcal strains. However, it also clearly shows that under laboratory conditions there is no direct relationship between the high virulence of gonococci for man and their infectivity for guinea-pig chambers. On the contrary, a strain which may be non-infective for the guinea-pig chamber, may have shown marked invasiveness for man. From this, it follows that the artificial increase in virulence of gonococcal isolates for the guinea-pig chamber (Arko *et al.*, 1976b; Penn *et al.*, 1977a), although interesting from the point of view of experimental infection, may not be directly comparable with the natural condition in man.

The current data indicate that non-infectivity for the guinea-pig chamber cannot yet be regarded as an irrefutable indicator of DGI properties, since some prototrophic strains were also found to be non-infective. Although on rare occasions, prototrophic strains have been obtained from disseminated gonococcal infection (Knapp and Holmes, 1975), as yet, no serious attempt has been made to distinguish them from the usual gonococcal strains using laboratory criteria. It also appears that although non-infectivity for the guinea-pig chamber is very often accompanied by specific growth requirements, these properties may occur independently. It should be possible to determine their relationship using genetic analysis.

Table 4 *Agglutination titres of immunised guinea-pig sera against the homologous gonococcal strain. Serum samples taken 10–14 days after challenge*

Suppliers of Dunkin-Hartley guinea-pigs	Total no. of guinea-pigs	Reciprocal of agglutination titres			
		0	2–8	16–24	> 28
Charles River	54	2*	20	19	13
Olac	66	9	8	17	32

*No. of guinea-pigs per titre group

The finding that the properties of gonococcal strains can be determined experimentally *in vivo* has broadened the usefulness of the guinea-pig infectious model. On the other hand, however, its use in studies of immunological phenomena appears to be limited to the nutritionally less demanding strains—such as, those strains prevalent in uncomplicated symptomatic gonorrhoea. Despite this limitation, it would appear foolish to abandon this model in studies concerning the immunological and pathophysiological phenomena of these gonococci. Although the model is based upon the infection of an artificial cavity lined with fibrous tissue and not by mucosal epithelium, a characteristic inflammatory reaction develops (Chandler *et al.*, 1976; Novotny *et al.*, 1977). From the onset of the gonococcal infection, the cellular reaction has proliferative qualities which anatomically determine the chronic character of the infection (Novotny *et al.*, 1977). It is this chronicity which bears the closest resemblance to the untreated disease in man (Chandler *et al.*, 1976).

We are unable to explain why some breeds of guinea-pigs develop a high immune resistance after immunisation, whereas in other breeds the immune resistance is either weak or completely absent, thus rendering the size of the challenge critical. However, even with very low challenges (50 cfu), Penn *et al.* (1977b) were unable to confer protection in Olac-bred guinea-pigs which had been immunised with five doses of gonococcal vaccines. On the other hand, Olac guinea-pigs are used in the Quality Control Department in these laboratories, and have been found suitable for the routine testing of several toxoids and whole cell vaccines and are, in these respects, comparable with guinea-pigs supplied by other breeders (Mr P. Knight, personal communication). It appears, therefore, that the differences observed in these experiments with gonococci are a direct consequence of the chamber model itself rather than of any general deficiency in the guinea-pigs. It is therefore apparent that some form of standardisation in the use of the chamber model is necessary if the results from different laboratories are to be compared. However, it is questionable whether any time devoted to an investigation into the immune resistance of the various lines of guinea-pigs would be relevant to our understanding of the disease in man.

We are grateful to Miss C. Briselden, Miss F. Smith, and Mrs B. Spacey for excellent technical assistance, and are deeply indebted to Professor B. W. Catlin (The Medical College of Wisconsin) and Dr R. J. Arko (CDC, Atlanta) for their advice on agar detoxification and gonococcal agglutination.

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Serological diagnosis of gonorrhoea by an enzyme-linked immunosorbent assay (Elisa)

A. A. GLYNN AND CATHERINE ISON

From the Department of Bacteriology, Wright-Fleming Institute,
St Mary's Hospital Medical School, London

SUMMARY IgG antibody to an outer membrane protein extracted from *Neisseria gonorrhoeae* was measured in patients with gonorrhoea. The level in such patients was significantly higher than in normal controls or in patients with other conditions who were attending the clinic for sexually transmitted diseases. Significantly higher antibody levels were detectable in half the patients within a few days of infection and in a similar proportion of carriers—both male and female. Men with rectal gonorrhoea had particularly high antibody levels. Sixteen per cent of presumptively normal men and 11% of normal women gave positive results but the actual false positive rate could be lower.

Introduction

Despite widespread efforts, especially recently, there is still no satisfactory serological test for diagnosing gonorrhoea (Watt *et al.*, 1971). The ultimate criterion of gonococcal infection is that *Neisseria gonorrhoeae* can be isolated from a patient, and this will remain the criterion. The difficulties in culturing gonococci and the problems of obtaining satisfactory specimens for culture make it desirable that an accurate method of serodiagnosis be available for individuals, particularly for screening populations.

The specificity of a serological test is usually inversely related to its sensitivity, but modern methods of antibody detection have greatly increased the general level of sensitivity at which this paradox occurs. It is essential, therefore, to find a suitable antigen. Because of its chemical stability, and hence ease of preparation, gonococcal lipopolysaccharide has often been used (Maeland and Larsen, 1971; Ward and Glynn, 1972).

However, the number of antigenically determinant sugars in gonococcal lipopolysaccharide is small (Perry *et al.*, 1975; Stead *et al.*, 1975), which may explain why positive reactions are sometimes found in uninfected people, presumably owing to cross-reacting antibody formed against non-pathogenic

Neisseria and other organisms (Glynn and Ward, 1970).

Gonococcal pili are much more specific antigens and the radioimmunoassay for antibody to pili introduced by Buchanan *et al.* (1973) is potentially of great value, although it has so far been evaluated only to a limited extent (Oates *et al.*, 1977).

The preliminary findings of an investigation into the use of an enzyme-linked immunosorbent assay (Elisa) to detect antibodies to an outer membrane protein of *N. gonorrhoeae* in patients with gonorrhoea are reported.

Methods

POPULATIONS

Sera were obtained from six groups of people (Table 1). The gonococcal group consisted of patients attending the clinic for sexually transmitted diseases (STDs) from whom *N. gonorrhoeae* had been identified by culture or smear or both. The non-gonococcal group comprised patients attending the same clinic but from whom no *N. gonorrhoeae* had been identified. Some had non-specific urethritis or herpes infection, some were simply anxious, and others were being followed-up from an earlier attack of gonorrhoea (Table 6).

Cultures were taken from the cervix of all female patients; in the males all urethral exudates were examined microscopically but only 45 were cultured. The reason for culture in men varied, but it was often the presence of rectal symptoms or some unusual

Address for reprints: Professor A. A. Glynn, Department of Bacteriology, Wright-Fleming Institute, St Mary's Hospital Medical School, London W2 1PG

Received for publication 21 September 1977

Table 1 *Populations studied*

Group	Men			Women			Total		
	No.	Age (years)		No.	Age (years)		No.	Age (years)	
		Mean	Range		Mean	Range		Mean	Range
Normal controls	106	21	18-36	131	24	15-43	237	23	15-43
Patients without gonorrhoea	84	31	17-61	94	26	16-61	178	28	16-61
Patients with gonorrhoea	191	30	17-56	212	23	15-50	403	26	15-56
Total	381	28	17-61	437	24	15-61	818	26	15-61

feature. It is not possible, therefore, to draw any general conclusions from this group on the relationship between cultural and smear results in straightforward urethritis.

The sera used were from those sent routinely for serological tests for syphilis. All the sera from patients with proved gonorrhoea were taken on given days for a period of several months. During a further period all sera sent from the STD clinic were examined.

Normal control sera were from patients attending the antenatal clinic and from male and female students to whom the purpose of the investigation had been explained. About half the students approached agreed to take part. As individual results would not be disclosed, there was no reason why a student who had had gonorrhoea should not have been willing to volunteer although some may have refrained.

PREPARATION OF ANTIGEN

Antigen was prepared from a recently isolated strain of *N. gonorrhoeae* (H 1) stored in liquid nitrogen. Subcultures from this were first grown on GC agar plates containing 36 g/l GC base agar (Difco) plus 20 ml/l Supplement A, containing 20% dextrose (BDH), 0.25% α -glutamine (Sigma), and 0.002% co-carboxylase (Sigma) plus 2 ml/l Supplement B, containing 0.5% ferric nitrate (Sigma). The bacteria harvested from the plates were used to seed a biphasic system (description of method to be published) consisting of dextrose starch agar (Difco), 65 g/l, reinforced by Bacto-agar (Difco), 10 g/l, covered by proteose peptone (Difco) plus Supplements A and B, and shaken for six or seven hours.

The growth from all flasks was checked for purity by Gram's stain, harvested by centrifugation at 6000 $\times g$ for 15 minutes, pooled and washed twice in phosphate buffered saline (PBS), and again centrifuged. The wet weight was recorded and the pellet stored at -20°C .

An outer membrane protein antigen was prepared as described by Smyth *et al.* (1976). Twenty grams wet weight of *N. gonorrhoeae* were lysed, the cell

envelopes separated by centrifugation and extracted with TritonX100 followed by desoxycholate. The desoxycholate fraction was used and will be referred to hereafter as the antigen. The antigen was stored in 100 μl amounts at -20°C .

PREPARATION OF ALKALINE

PHOSPHATASE ANTIHUMAN IgG CONJUGATE

Rabbit antihuman IgG serum was precipitated with 18% followed by 12% anhydrous sodium sulphate. The precipitate was dissolved in the smallest volume possible and the IgG fraction separated on a DEAE cellulose column.

After concentration using an Amicon filter (PM 30 size 25 mm), the rabbit antibody was conjugated with alkaline phosphatase (Sigma type 7) in a 2:5 ratio as described by Voller *et al.* (1976).

ASSAY PROCEDURE

One hundred microlitres of antigen diluted in carbonate-bicarbonate buffer (pH 9.6) were added to each well in a flat-bottomed microtitre plate (Cooke M29AR, Dynatek Laboratories), using a Compu-pet 100 (General Diagnostic), and incubated at room temperature for 30 minutes. The antigen was then tipped out and the plates were washed by refilling with PBS-Tween (PBS pH 7.4 + 0.05% Tween 20). This was left for three minutes, and the washing repeated three times. The test sera were diluted 1:1000 in PBS-Tween, and 200 μl of each were added to an antigen-coated tray. Each serum was tested in duplicate. Trays were incubated in moist conditions for one hour at 30°C . They were then washed three times in PBS-Tween as before.

The antihuman IgG alkaline phosphatase conjugate was diluted to its working strength in PBS-Tween, 200 μl were added to each well, and the trays incubated for two hours.

The trays were again washed three times in PBS-Tween and 200 μl substrate (1 mg/ml p-nitrophenyl phosphate (Sigma)) in 10% diethanolamine (BDH) buffer (pH 9.8) were added to each well for exactly 30 minutes. The reaction was stopped by adding 50 μl of 1 mol/l sodium hydroxide and the colour reaction in each well was read at 405 nm.

Samples of a negative and positive control serum were tested in duplicate in each tray.

The negative control serum was a pool of sera from five male and five female students. The positive control was taken from a patient with gonococcal arthritis and it was used at a dilution of 1 in 2000.

Results were expressed as corrected extinction values E_{cr} calculated as

$$E_{cr} = (E_t - E_n) \times \frac{\text{mean } (E_p - E_n)}{(E_p - E_n) \text{ on test day}} \times 100$$

where E_t , E_p , and E_n are the extinction values ($\lambda = 405$ nm) of the unknown and positive and negative control sera. The mean $(E_p - E_n)$ is the mean difference between the positive and negative control sera measured in 20 assays during a period of months.

STANDARDISATION OF ANTIBODY ASSAY

It is important in the Elisa technique that there should be an excess of antigen. Dilutions of known positive and negative sera were tested against increasing antigen concentrations. In the example shown (Fig. 1), a plateau was reached at concentrations of antigen above 20 μg protein/ml. The actual concentration of antigen varied with the batch of antigen and its age but it was always in the plateau range. For each new preparation of antigen the concentration was adjusted so as to keep the difference constant between the standard control positive and negative sera. Similar adjustments were made to the concentration of each new batch of enzyme-conjugated antihuman IgG.

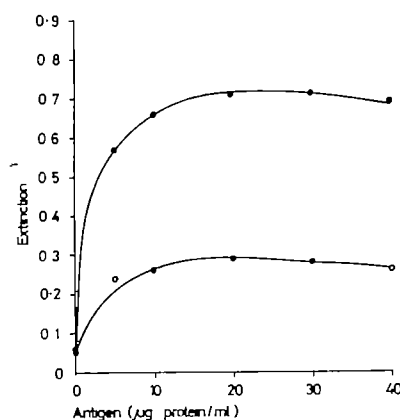


Fig. 1 Effect of antigen concentration on extinction (antibody level).

Positive control serum ●—●
Negative control serum ○—○

For routine tests a standard dilution of unknown sera of 1:1000 was chosen (Fig. 2). This gave a small but readable extinction value with normal sera and it still allowed most positive sera to be read without further dilution—that is, to give E_{cr} of less than 1.0.

Fourteen test sera plus the sera from positive and negative controls were assayed 20 times during a period of a week. The results showed a 20% coefficient of variation. Variation between days was greater than variation between different trays done on the same day.

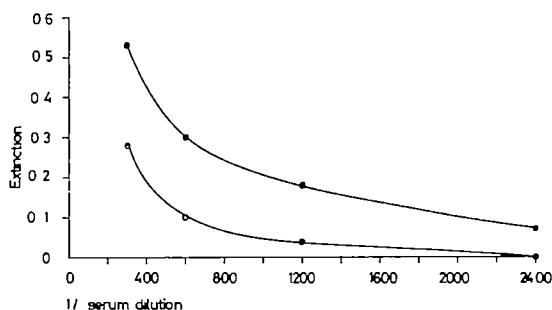


Fig. 2 Effect of diluting serum on extinction.

Positive control serum ●—●
Negative control serum ○—○

ANALYSIS OF RESULTS

The results were analysed with the help of a CDC 6600 computer at the University of London computer centre using statistical and other programs from the Statistical Package for the Social Sciences (SPSS) (Nie *et al.*, 1975).

Student's *t* test was used for Table 2 and an analysis of variance for Table 6.

Results

POPULATIONS

The number of people in each group and their age distribution is given in Table 1. Although there are some differences in age distribution, in no group was antibody activity affected by age.

ANTIBODY ACTIVITY BY GROUPS

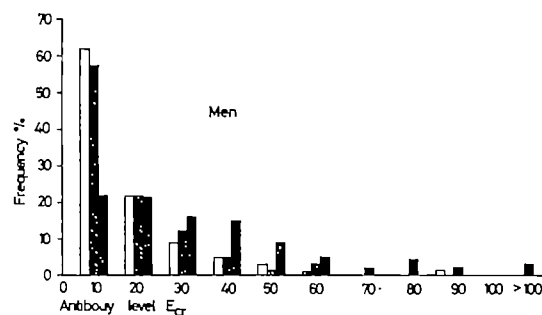
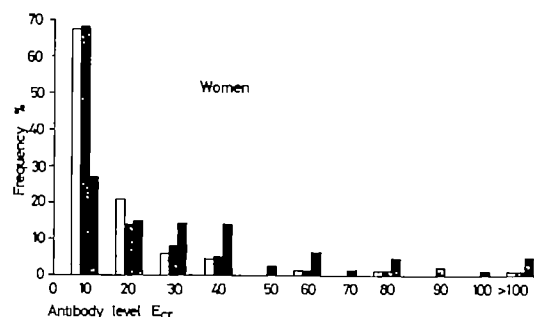
There were no significant differences in mean antibody levels between men and women in comparable groups, but for either sex the mean for patients with gonorrhoea was significantly higher than that for control subjects (Table 2). In the normal groups the frequency distribution (Figs 3 and 4) showed marked clumping at the bottom of the scale with a few isolated high readings. The antibody levels in patients

Table 2 Antibody activity (E_{cr}) by groups

Group	Men		Women	
	Mean E_{cr}	(SE)	Mean E_{cr}	(SE)
Normal controls	10.1 ^a	(1.3)	10.0 ^b	(1.8)
Patients without gonorrhoea	11.8 ^c	(1.3)	11.4 ^d	(1.8)
Patients with gonorrhoea	30.5 ^e	(2.4)	34.5 ^f	(2.7)

Student's *t* tests

Comparing ab, cd, cf, ac, bd not significant

 ae
 ce
 bf
 df
 $P < 0.0001$
Fig. 3 Frequency distribution of antibody levels (E_{cr} units) in men.
 Normal controls □
 Patients with conditions other than gonorrhoea ▨
 Patients with gonorrhoea ■
Fig. 4 Frequency distribution of antibody levels (E_{cr} units) in women.
 Normal controls □
 Patients with conditions other than gonorrhoea ▨
 Patients with gonorrhoea ■

with gonorrhoea were more evenly distributed across the whole range.

In both sexes the patients with conditions other than gonorrhoea from the STD clinic did not differ significantly in means or in frequency distribution from the normal controls.

ANTIBODY LEVELS IN INDIVIDUALS

Although the gonococcal patients as a group had significantly higher antibody levels than the normal subjects, the two groups overlapped. Whereas means are useful to compare populations, the clinical interpretation of antibody level in an individual patient is best considered in relation to the cumulative frequencies (Table 3). If an antibody level of 15 or more E_{cr} units is taken as abnormal, 16% of our normal male population would give false positive results and 11% would do so in the female.

This percentage could be reduced to 8.5% for both sexes if the abnormal range was raised to 20 or more E_{cr} units, but after that the reduction would be negligible. The 'false' positive results may be caused by extreme variation in a normal population, perhaps the result of stimulation by cross-reacting antigens. However it is possible, even likely, that some at least are owing to unsuspected or undeclared gonorrhoea in a normal population.

Depending on whether 14 or 19 E_{cr} units is taken as the limit, just over or just under half the patients with proved gonorrhoea would be regarded as having an antibody response.

Table 3 Cumulative frequency (%) of patients by groups at different antibody levels

		Cumulative frequency (%)					
		Patients					
Antibody (E_{cr})	Normal controls	Without gonorrhoea		With gonorrhoea			
		Men	Women	Men	Women	Men	Women
≥10-14	23	16	25	24	64	63	
≥15-19	16	11	21	18	56	55	
≥20-24	8	8	14	12	47	47	
≥25-29	7	6	9	10	39	41	
≥30-34	6	3	6	6	31	32	

EFFECT OF DURATION OF INFECTION ON ANTIBODY

Of the men with gonorrhoea, 128 (88%) had had the disease for a week or less and 98 (67%) for three days or less. The mean antibody level rose markedly and significantly with time ($P < 0.0001$). Conversely the numbers of false negatives fell from 51% during the first three days to 33% during the second half of the week. The pattern suggests a rapid antibody response, falling away within a week or two of treatment which was usually started on the patient's first attendance (Table 4).

Among women the pattern was different. There was no significant relationship between antibody level and duration although there was some indica-

tion of a decrease after two or three weeks. Women were generally not seen as early as men (only 60% in the first week) and the figures may well underestimate the duration of infection (Table 5).

OTHER FACTORS AFFECTING THE ANTIBODY LEVEL (Table 6)

In both sexes asymptomatic carriers had the same mean antibody levels as patients with gonorrhoea. The level was significantly higher in men with

gonorrhoea who had rectal symptoms and in men with positive results to serological tests for syphilis. This finding is probably a reflection of the high incidence of syphilis among male homosexuals attending the clinic; it was not seen in the small group of women with positive results to serological tests for syphilis.

Antibody was also very high (60 E_{50} units) in two women with disseminated gonococcal infection.

Among patients without gonococcal infection the antibody level was higher if there was a history of gonorrhoea. This difference was greater in men than in women. Further analysis of the men showed that higher antibody levels were related to a previous attack of gonorrhoea within the past 12 weeks. The half-life of human IgG is 23 days so that if no further antibody was made once the infection was over, the antibody level would drop approximately 16-fold in 12 weeks. The figures also suggest that if any of the raised antibody levels found among the control subjects were in fact caused by gonorrhoea, it must have been a recent attack.

Among patients with gonorrhoea, antibody levels were not affected by a past attack. It is possible that any residual effect of a recent infection would be swamped by the antibody response of the current one.

Discussion

The Elisa technique worked smoothly and with practice one person could easily test 100 sera a day. Moreover the test could be calibrated so that the results are expressed as μ g of antibody/ml serum.

Table 4 *Effect of duration of present attack of gonorrhoea on antibody levels in men*

Duration (days)	No.	% with antibody <20 E_{50} /ml	Mean antibody level (E_{50} /ml)
1-3	98	51	23
4-7	30	33	32
1-7	128	47	25
8 and more	17	24	49
Subtotal	145	44	28
Not known	46	41	36
Total	191	43	30

*Equivalent to % false negative

Table 5 *Effect of duration of present attack of gonorrhoea on antibody levels in women*

Duration (days)	No.	% with antibody <20 E_{50} /ml	Mean antibody level (E_{50} /ml)
1-3	32	44	34
4-7	26	42	36
1-7	58	43	35
8 and more	38	42	25
Subtotal	96	43	32
Not known	116	47	36
Total	212	45	34

*Equivalent to % false negative

Table 6 *Factors affecting antibody levels*

Factor	Male patients				Female patients			
	With gonorrhoea		Without gonorrhoea		With gonorrhoea		Without gonorrhoea	
	No.	Mean E_{50}	No.	Mean E_{50}	No.	Mean E_{50}	No.	Mean E_{50}
Asymptomatic carriers	19	28	—	—	64	30	—	—
Symptoms	168	28	—	—	143	33	—	—
Rectal symptoms								
Yes	23	39 ^a	—	—	—	—	—	—
No	168	27 ^b	—	—	—	—	—	—
Serological test for syphilis								
Positive	24	42 ^a	18	16	8	24	5	5
Negative	159	26 ^a	62	10	195	32	84	12
Doubtful	7	29	3	16	8	41	5	2
History of gonorrhoea								
Yes	98	28	36	15 ^a	50	34	18	15
No	62	27	26	8 ^a	118	30	62	10
History of sexually transmitted disease unspecified	15	31	11	14	14	47	6	2
Time since last attack								
<12 weeks	—	—	9	24 ^a	—	—	—	—
>12 weeks	—	—	20	11 ^b	—	—	—	—
Total in group	191		84		212		94	

Comparing a b $P=0.02$, c d $P=0.01$, e f $P=0.025$, g h $P=0.004$. Other differences are not statistically different

The variability was still more than one would wish but less than that of classical double dilution titrations. The largest part of the error could be attributed to differences in the amounts of antigen sticking to the microtitre plates. The antigen contained many membrane proteins. Further purification and definition of specificity are needed.

The early stage of infection at which most sera were taken accounted for the inability to detect no more than 57% of the men with proved gonorrhoea but it would have had less effect in the women. Increasing the precision of the test and checking the quality of the normal controls should improve the yield.

The results differed from those of other tests (Ward and Glynn, 1972; Buchanan *et al.*, 1973; Oates *et al.*, 1977) in the closeness of antibody levels in men and women, both in cases and in carriers. The ability to pick up symptomless male carriers is particularly interesting. Generally the results primarily indicate a current infection. A previous infection had a slight modifying effect but only if it was recent. If an antibody level of over 15 E_{cr} was taken as indicating the presence of gonococci, there would be an 84% chance of being right in men and an 89% chance in women. This is based on the findings in the normal population. If the same figures were applied to patients attending the STD clinic with conditions other than gonorrhoea, they would give a minimum 5% failure rate in the detection of gonococci by smear and culture. Even as it is at present, the test could help to diagnose cases, and to a lesser extent it could be used in screening.

Preliminary results have also shown the presence of IgM antibody although in smaller quantities than IgG. It is perhaps relevant that Cohen *et al.* (1969) using a fluorescence technique detected predominantly IgG antibodies to a surface, labile, gonococcal antigen in experimentally infected volunteers. This antibody appeared at five to seven days, reached a peak at 14, and then fell. In contrast antibody to gonococcal lipopolysaccharide was predominantly IgM in character (Cohen *et al.*, 1969; Maeland and Larsen, 1971). Apart from

duration, the special clinical features associated with higher antibody levels were the presence of rectal symptoms or disseminated infection.

Dr R. R. Willcox has given us encouragement and help over a long period. We thank him, Dr J. R. W. Harris, and the staff of the Praed Street Clinic for allowing us access to their patients and for collecting the blood. We thank the patients, students, and others who willingly gave blood, Miss Angela Evans for her technical assistance, and Dr H. Gaya and Mr J. Thirlwall for instruction, help, and advice with the computer analysis.

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The antimicrobial susceptibility of *Chlamydia trachomatis* in cell culture

G. L. RIDGWAY, J. M. OWEN, AND J. D. ORIEL

From the Departments of Clinical Microbiology and Genitourinary Medicine,
University College Hospital, London

SUMMARY The action of 22 antimicrobial agents against the SA₂f strain of *Chlamydia trachomatis* has been studied by the use of a simple cell culture technique. Tests for bactericidal activity were undertaken with some of the agents, and latency of infection was demonstrated. The susceptibilities of 10 clinical strains of *C. trachomatis* and of SA₂f to oxytetracycline, erythromycin, and spectinomycin in cell culture were found to be identical.

Introduction

The importance of *Chlamydia trachomatis* as a pathogen in the human genital tract is now well recognised, but the study of the action of antimicrobial agents against *Chlamydia in vitro*, an essential preliminary to rational treatment, was long hampered by the lack of a simple and reliable experimental system. Early work with embryonated chicken egg yolk sacs and with animal models (Werner, 1961) yielded valuable data, but the methods were slow and cumbersome. Recently, however, advances in cell culture systems (Gordon and Quan, 1965; Wentworth and Alexander, 1974) have made the *in vitro* testing of antimicrobial drugs against *Chlamydia* much easier. We have described a technique with which eight drugs were tested against a laboratory-maintained strain of *C. trachomatis* (SA₂f) in McCoy cell cultures (Ridgway *et al.*, 1976), and Kuo *et al.* (1977) investigated the action of seven drugs against the 15 known immunotypes of *C. trachomatis* in HeLa 229 cell cultures.

In the experiments reported here we have extended our earlier study in three directions: (1) We have estimated the minimum inhibitory concentration (MIC) of 22 antimicrobial agents against SA₂f. As before, MIC is defined as the lowest concentration of drug preventing the appearance of any chlamydial inclusions in the cell monolayers. (2) The minimum cidal concentration (MCC) of eight agents against SA₂f was estimated. MCC is defined as the minimum

concentration of drug required to prevent further infection of the cell monolayers, even after multiple passage. *C. trachomatis* infections exhibit latency (Hanna *et al.*, 1968), so for any particular drug the MIC and MCC may not necessarily be the same. (3) To see whether experience derived from studies with SA₂f can be applied to clinical isolates of *C. trachomatis*, the MIC of various antimicrobial agents against SA₂f and against 10 freshly isolated strains of *Chlamydia* were compared.

Materials and methods

The organism used for most of this work was the SA₂f strain of *C. trachomatis*, but some experiments were also done with low passage isolates from patients with non-gonococcal urethritis or cervicitis.

DETERMINATION OF MIC

The method has been described elsewhere (Ridgway *et al.*, 1976). Briefly, doubling dilutions of the antimicrobial agent were prepared in agent-free *Chlamydia* growth medium. One millilitre of each dilution was added to a flat-bottomed plastic tube containing a coverslip monolayer of 5-iodo-2-deoxyuridine (IUDR) treated McCoy cells. A suspension of *Chlamydia* containing approximately 200 inclusion-forming units per ml was added to each tube. After centrifugation at 3000 *g* for an hour, the tubes were incubated for between 38 and 40 hours at 35°C. The coverslips were removed and the cell monolayer was fixed with methanol and stained either with Giemsa for examination of auto-fluorescence using darkground illumination, or with iodine for examination of stained inclusions by brightfield illumination. Both methods of staining

Address for reprints: G. L. Ridgway, Department of Clinical Microbiology and Genitourinary Medicine, University College Hospital, London WC1

Received for publication 13 October 1977

were used in case some of the antimicrobial agents affected only one, as is known to happen, for example, with penicillins which inhibit glycogen production with the consequent formation of inclusions which stain poorly with iodine (Jawetz, 1969). The MIC was taken as the lowest concentration containing no inclusions with either staining method.

DETERMINATION OF MCC

Four tubes containing IUDR-treated McCoy cells were prepared for each dilution of antimicrobial drug, and inoculated and incubated as above. After incubation, the coverslips from two tubes were fixed and stained, one with Giemsa and the other with iodine. The medium was removed from the remaining two tubes and replaced with 2 ml of medium free from antimicrobial drugs. The cells were rubbed off the coverslip with a sterile rubber-tipped glass rod, resuspended in the medium, and used to inoculate two further tubes containing fresh coverslip monolayers of IUDR-treated McCoy cells. Thus the contents of the two tubes were passaged into four tubes. After centrifugation, tubes were incubated in the usual manner. This passage procedure was repeated as required, and the MCC was taken as the lowest concentration of antimicrobial agent preventing demonstrable inclusions after 10 passages.

Strict precautions were taken to prevent cross-contamination with *Chlamydia*. No other work with this organism was undertaken while passage experiments were being performed, safety cabinets were always used, and work proceeded from higher to lower concentrations of antimicrobial agent.

Results

MIC

Table 1 shows the MIC of various antimicrobial agents to the SA₁f strain. For all drugs except

penicillins, cephalosporins, and folic acid antagonists (sulphamethoxazole and trimethoprim) the end point was sharp, and was the same whether Giemsa or iodine staining was used. Penicillins, cephalosporins, and the folic acid antagonists produced abnormal forms which often persisted throughout the dilutions tested. The MIC of these drugs was taken to be the lowest concentration which showed no autofluorescent inclusions on darkground examination; this coincided with the predominance of the abnormal forms over the normal inclusions. To determine whether the abnormal inclusions were viable, multiple passage was performed with some agents.

MCC

A typical result of a test for the MCC of an antimicrobial agent is shown for sulphamethoxazole in Table 2. In this case, no tubes yielded inclusions after the sixth passage. It will be seen that several 'blind' passages were necessary before inclusions could be seen by light microscopy in tubes containing concentrations below the MCC. The results for all the drugs tested by passage are shown in Table 3. The number of passages required before no further tubes yielded inclusions varied between drugs, but was constant for each.

CLINICAL ISOLATES

The action of oxytetracycline, erythromycin, and spectinomycin was assessed against 10 strains of *C. trachomatis* recovered from patients with NGU or cervicitis, using SA₁f as a control. The results are shown in Table 4. There was no marked difference between the results obtained with these isolates and with SA₁f.

Discussion

SA₁f is immunologically identical with the LGV II serotype (Wang and Grayston, 1970), which cross-reacts with Groups B and ED, immunotypes

Table 1 *Minimal inhibitory concentrations (MIC) of various antimicrobial agents against Chlamydia trachomatis (SA₁f) in cell culture*

High activity		Medium activity		Low activity	
Antimicrobial	MIC (µg/ml)	Antimicrobial	MIC (µg/ml)	Antimicrobial	MIC (µg/ml)
Rifampicin	0.007	Ampicillin	0.25	Spectinomycin	64
Doxycycline	0.03	Mecillinam	0.25	Oxolinic acid	128
Minocycline	0.03	Penicillin	1.0	Trimethoprim	128
Oxytetracycline	0.06	Clindamycin	1.0	Cefuroxime	256
Erythromycin (base and lactobionate)	0.06	Rifamide	1.0	Lincomycin	512
		Amoxycillin	2.0	Vancomycin	>256
		Cephaloridine	2.0	Metronidazole	>256
		Sulphamethoxazole	4.0	Gentamicin	>512
		Chloramphenicol	4.0		

Table 2 Recovery of *Chlamydia trachomatis* (SA₂f) after initial incubation with sulphamethoxazole

Concentration of sulphamethoxazole (µg/ml)	Passage number										
	0	1	2	3	4	5	6	7	9	9	10
1.0	+	—	+/-	+/-	+	ND	ND	ND	ND	ND	ND
2.0	+	—	—	—	+	ND	ND	ND	ND	ND	ND
4.0	(-)	—	—	—	—	+	ND	ND	ND	ND	ND
8.0	(-)	—	—	—	—	—	+	ND	ND	ND	ND
16.0	(-)	—	—	—	—	—	+	ND	ND	ND	ND
32.0	(-)	—	—	—	—	—	—	—	—	—	—

+ Giemsa-stained autofluorescent inclusions

(-) Autofluorescence absent; tiny abnormal inclusions present

— Inclusions not seen by light microscopy

ND Not done

MIC and MCC of sulphamethoxazole in this experiment were 4 µg/ml and 32 µg/ml respectively

Table 3 Relationship between minimum inhibitory concentration and minimum cidal concentration for various antimicrobial agents against *Chlamydia trachomatis* (SA₂f)

Antimicrobial agent	Minimum inhibitory concentration (µg/ml)	Minimum cidal concentration (µg/ml)	No. of passages required for cidal end-point
Oxytetracycline	0.06	0.25	2
Erythromycin	0.06	0.5	3
Mecillinam	0.25	> 1.0	2
Cephaloridine	2.0	> 32.0	3
Sulphamethoxazole	4.0	32.0	6
Spectinomycin	64	128	1
Trimethoprim	128	1024	3
Cefuroxime	256	> 512	1

Table 4 Minimum inhibitory concentrations of various antimicrobial agents to clinical isolates of *Chlamydia trachomatis* in cell culture

Strain no.	Serotype	Oxytetracycline (µg/ml)	Erythromycin (µg/ml)	Spectinomycin (µg/ml)
1	E	0.03	0.06	32
2	DE	0.03	0.03	64
3	DE	0.06	0.03	32
4	G	0.06	0.12	64
5	DE	0.06	0.06	64
6	D	0.03	0.03	64
7	D	0.06	0.03	64
8	D	0.06	0.03	64
9	D	0.06	0.03	64
10	D	0.06	0.03	64
SA ₂ f	LGV II	0.06	0.06	64

commonly associated with human genital infections. In the present study the MICs of oxytetracycline, erythromycin, and streptomycin against 10 genital strains and SA₂f were closely similar. All but one of these clinical isolates were in the DE complex of immunotypes, reflecting their genital origin, but Kuo *et al.* (1977), who tested all the 15 known immunotypes of *C. trachomatis*, found only minor differences between their responses to tetracycline and erythromycin. Thus, SA₂f appears to be a suitable organism in evaluating the anti-chlamydial action of antimicrobial drugs for use in genital tract infections, and it also has the advantages of being readily available and safe to laboratory workers.

Rifampicin was the most active of the drugs tested, but it has not been evaluated clinically for the treatment of genital tract infections. The tetracyclines and erythromycin are both highly active against *Chlamydia*, as earlier work with laboratory models has shown (Jawetz, 1969). There was a marked difference between the MICs of lincomycin and clindamycin, between rifampicin and rifamide, and between cephaloridine and cefuroxime. It is possible that these differences are owing to variations in the cell-penetrating properties of the antibiotics. The activity of related antimicrobial agents evidently cannot be predicted from the results obtained for one member of the group.

With some drugs there was a considerable difference between the MIC and the MCC. This was particularly apparent with the cell wall inhibitors, mecillinam and cephaloridine, and with the folic acid antagonists, sulphamethoxazole and trimethoprim. It is well known that cell wall inhibitors, such as penicillin, promote the formation of abnormal chlamydial inclusions. Johnson and Hobson (1977) were unable to render these abnormal forms viable by replacing the penicillin-containing medium with penicillin-free medium, without passage. However, Kuo *et al.* (1977), using single passage, demonstrated large differences between MIC values for infectivity and for viability of both penicillin and ampicillin against strains of *C. trachomatis*. Our own studies with mecillinam indicate that these inclusions readily recover their infectivity on passage on to fresh cell monolayers in medium free from antimicrobial drugs, but that multiple passage may be necessary.

The data summarised in Table 3 indicate that after the action of some agents it may be impossible to see chlamydial inclusions by microscopical examination, although after passage normal infectious inclusions may reappear. These observations suggest that within the system described here some antimicrobial drugs induce latency. In ocular chlamydial infections latency is thought to be common (Hanna *et al.*, 1968), and it has been postulated that this is so for genital tract infections (Richmond *et al.*, 1972), but latency with *C. trachomatis* has not previously been demonstrated *in vitro*.

MIC estimations broadly indicate which compounds are likely to be effective in the treatment of genital chlamydial infection; however, the results should be interpreted with caution, as *in vitro* the cidal concentration may differ considerably from the inhibitory concentration. Furthermore, if latency can be induced by antimicrobial drugs *in vivo* as well

as *in vitro*, the concept of cure of these infections may require re-evaluation, and longer periods of follow-up after treatment than are currently practised may be advisable.

We are grateful to Dr E. Joan Stokes for kindly providing laboratory facilities, to Ms V. Lam Po Tang for technical assistance, and to Ms P. M. Waterworth for advice. Dr Brenda Thomas, Clinical Research Centre, Harrow, Middlesex, kindly undertook the immunotyping of the clinical isolates.

This work was supported by a grant from the Medical Research Council.

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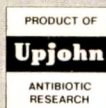
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1. *Current Medical Research and Opinion*, 3, 83-88, 1975. 2. *British Journal of Venereal Diseases*, 53, 126-128, 1977. Canesten is available as vaginal tablets, each containing 0.1g clotrimazole, in packets of six with applicator and as cream, containing 1% clotrimazole, in 50g and 20g tubes. Canesten Duopak contains six vaginal tablets with applicator plus 20g cream. Further information including data sheet is available from Bayer U.K. Limited, Pharmaceutical Division, Haywards Heath, West Sussex, RH16 1TP. Telephone: Haywards Heath (0444) 57911. ® Registered Trade Mark of Bayer Germany



Treatment and prognosis of non-specific genital infection

B. A. EVANS

From the Department of Genitourinary Medicine, West London Hospital

SUMMARY In a comparative trial minocycline was the best oral tetracycline compound for twice-daily administration in the treatment of non-specific urethritis. A review six months later showed that 29% of patients had needed further treatment, 35% of these more than one month after initial treatment and 12% between three and six months later. The history revealed no evidence of predisposition to non-specific urethritis among patients in whom relapse occurred. Multiple relapses were in the groups of patients who relapsed earliest. These cases proved more resistant to subsequent treatment, but all patients were eventually cured. The records of female contacts showed that 29% were symptomatic, 44% had an abnormal appearance of the cervix, but only 11% had inflammatory changes detected on cytological smear. A previous report was confirmed that there was no apparent benefit from the empirical treatment of contacts; possible reasons for this are given.

Introduction

The cure rate for non-specific urethritis (NSU) is consistently lower than that for most other sexually transmitted diseases of known aetiology. In this trial two drugs which have been reported to be particularly effective are compared with low-dose oxytetracycline (250 mg twice daily). Minocycline (Minocin), 200 mg immediately and 100 mg twice daily for seven days, was found to have a three-month retreatment rate of 10.4% (Willcox *et al.*, 1975), and sustained-release oral tetracycline (Tetrabid-Organon), 500 mg immediately and 250 mg twice daily for seven days, is recorded as having a 1.3% failure rate four weeks after treatment (Perera, 1975).

A previous finding that as many as 10 (13%) out of 76 relapses occurred during the third month after treatment (Evans, 1977) led to the patients in the present trial being assessed after three months and again after six months, and also to multiple relapses being recorded. The value of empirical treatment of contacts has been re-examined, and the clinical and laboratory findings have been analysed in relation to relapse of urethritis.

Material and methods

This investigation was on 505 patients who attended between April and October 1976. Patients who had been treated for NSU during the previous 12 months were excluded, as were those who had been given antibiotics during the previous three months. Diagnosis of, or contact with, any other sexually transmissible disease also resulted in the patient being excluded from the trial, as did a history of tetracycline allergy.

Patients were allocated one of three treatments according to a prepared randomisation chart; this treatment remained unknown to the investigator until the outcome had been assessed. The regimens were oxytetracycline 250 mg twice daily, sustained release tetracycline (Tetrabid-Organon) one 250 mg capsule twice daily, and minocycline (Minocin) 100 mg twice daily; each was taken for seven days. Attendance for assessment was requested two weeks and four weeks after the start of treatment. The patients' documents were reviewed at three and six months.

Gram-stained smears of urethral exudate were examined with a $\times 100$ oil immersion objective lens, and the diagnosis of NSU was made if there were more than 10 polymorphonuclear leucocytes (PMN) in five microscope fields. Additional routine investigations were culture of urethral exudate for

Address for reprints: B. A. Evans, Department of Genitourinary Medicine, West London Hospital, Hammersmith Road, London W6 7DQ

Received for publication 8 September 1977

Neisseria gonorrhoeae and macroscopic examination of the first flush urine for urethral threads. Patients were regarded as cured if their urethral smear was normal after at least two hours' continence of urine. The outcome was assessed after three months and then again after six months from the start of treatment. Statistical significance was calculated by the χ^2 test. Contact slips were issued for the attendance of sexual partners.

Results

The 505 patients were aged between 16 and 62 years (mean 28); 23 (4.6%) were homosexual. Sixty-two patients (12%) were withdrawn from the trial for reasons that included gonorrhoea (seven cases), contact with trichomoniasis (five cases), and treponemal infection (11 cases, three of which were early syphilis). In most of the remainder the protocol had been ignored; 15 patients had been treated for NSU within the previous year and 129 (25%) patients did not complete two weeks' observation; so the result of treatment could be adequately assessed in only 314 patients. Table 1 gives an analysis of these results three months after treatment. A further 15

more than one occasion (Figure). There was no difference between the three treatment groups in this respect (Table 3). Subsequent treatment did not form part of the trial, but it was found that between 36% and 63% (mean 44%) of those who required further treatment had another relapse (Table 3). A history of NSU more than one year previously was given by 78 patients who were adequately assessed, and of these 43 were cured (23% of total cured) and 35 relapsed (27% of total who had relapses). Previous NSU did not correlate with unsuccessful treatment, and it did not correlate with repeated relapse (Table 4).

Patients for whom contacts had attended were grouped according to their own responses to treatment (Table 5). This shows no significant difference between the relapse and no relapse groups ($\chi^2=0.281$, $P>0.5$), and confirms the findings of an earlier paper (Evans, 1977).

Sixty-six female contacts of 64 patients attended and their clinical and laboratory findings are

Table 1 Outcome of trial at three months

Outcome	Drug group			Total patients	
	Oxytetracycline*	Tetracycline†	Minocycline	No.	%
Removed	19	24	19	62	12
Defaulted	41	40	48	129	25
Cured	67	60	73	200	40
Relapsed	42 (28%)	43 (30%)	29 (19%)	114 (26%)	23
Total	169	167	169	505	

*Low-dose †Sustained-release

patients relapsed during the next three months, but these have been omitted so that the results may be compared with a previous report (Evans, 1977) in which two other tetracycline compounds were compared with low-dose oxytetracycline and no difference in cure rate was found. In the present trial, minocycline produced the best results, with only 29 patients (19%) requiring further treatment during the next three months. During this period, 42 patients (28%) who received low-dose oxytetracycline and 43 patients (30%) who received sustained-release tetracycline required further treatment. Minocycline had approximately half the relapse rate of the other two tetracyclines during the period from two to four weeks after treatment when most relapses occur ($\chi^2=5.857$, $0.1>P>0.05$), but thereafter there was little difference in the relapse rates with the three drugs (Table 2).

After six months' observation 129 (29%) patients had relapsed, but 55 (12%) of them relapsed on

Table 2 Relapse of NSU during six months' observation

Period between treatment and relapse (weeks)	Drug group			Total patients	
	Oxytetracycline*	Tetracycline†	Minocycline	No.	%
2-4	36 (5)	30 (7)	18 (1)	84	65
5-13	6 (1)	13	11	30	23
14-26	7	3	5	15	12
Total	49	46	34	129	

Asymptomatic relapse shown in brackets

*Low-dose †Sustained-release

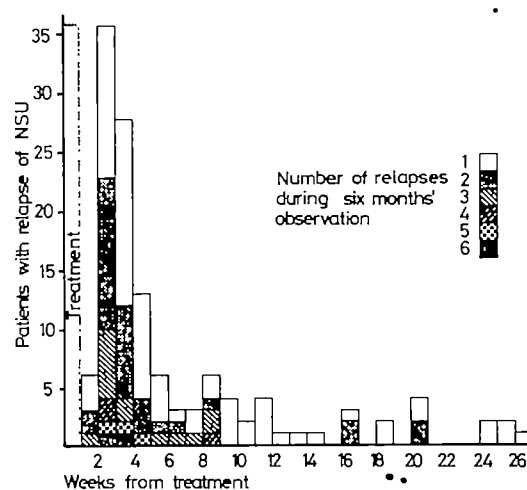


Figure Interval between initial treatment of NSU and diagnosis of relapse with infilling to show number of relapses during six months' observation

Table 3 Number of relapses during six months' observation

No. of relapses	Drug group			Total patients No. (%)	Relapse rate at each relapse %
	Oxytetracycline*	Tetracycline†	Minocycline		
1	24	29	21	74 (57.5)	43
2	16	10	7	33 (26)	40
3	6	5	3	14 (11)	36
4	1	1	1	3 (2)	63
5	2	0	1	3 (2)	40
6	0	1	1	2 (1.5)	
Total	49	46	34	129	

*Low-dose †Sustained-release

Table 4 Relapsing patients with a history of NSU

No. of relapses	Drug group			Total patients No. (%)
	Oxytetracycline*	Tetracycline†	Minocycline	
1	8	9	9	26 (74)
2	3	0	1	4 (11.5)
3	2	2	0	4 (11.5)
4	0	0	0	0
5	0	0	0	0
6	0	0	1	1 (3)
Total	13	11	11	35

*Low-dose †Sustained-release

Table 5 Treatment of contacts of patients with NSU

Patients with NSU	Relapse (weeks)			No relapse Total	
	2-4	5-13	14-26	No.	No.
Total	84	30	15	185	314
No. with a treated contact	22 (25%)	4 (13%)	3 (20%)	35 (19%)	64 (20%)
Delay before treatment (days)					
Mean	4.7	4.5	3.3	3.2	
Range	0-25	1-14	1-6	0-13	

summarised in Table 6. They are divided into two groups according to whether the patient with whom they were in contact relapsed after treatment or remained cured during the six-month period of observation. This was done on the principle that if a sexually transmitted organism is the cause of the disease, the contact of a patient in whom treatment proves unsuccessful is more likely to be infected than the contact of a patient who is cured. The groups were similar in respect of number of symptoms, contraception, appearance of cervix, purulence of cervical mucus under a microscope, and cervical cytology. The low incidence of inflam-

Table 6 Clinical and laboratory findings in 66 female contacts of NSU

Findings	Outcome of NSU treatment		
	Relapse (31 contacts)	No relapse (35 contacts)	Total contacts
	No. (%)	No. (%)	No. (%)
Symptoms			
No other infection	9 (29)	10 (29)	19 (29)
Candidosis	2	2	4
Gonorrhoea	—	1	1
Contraception			
Oral	17 (55)	25 (71)	42 (64)
Intrauterine device	5	3	8
Other	4	3	7
None	5	4	9
Cervical appearance abnormal	11 (35)	18 (51)	29 (44)
Mean pus cells in cervical mucus (1+10 per field; 2+20 per field)	1.48+	1.51+	1.50+
Cervical cytology			
Negative	25 (89)	31 (89)	56 (89)
NS inflammatory	3 (11)	4 (11)	7 (11)
Not done	3	0	3

matory changes in the cytological smears (11%) suggests that any micro-organism has little cytopathic effect.

Discussion

The value of a comparative trial is illustrated by the relapse rate for low-dose oxytetracycline in this trial compared with that reported previously (Evans, 1977). The corresponding figures are 19% for the trial carried out previously (Evans, 1977) and 28% for this. These figures illustrate the futility of comparing results obtained in trials undertaken on different populations at different times without the incorporation of a drug common to both trials. In this instance it is possible to compare the present results with those reported last year. The combined trials show that sustained-release tetracyclines (Sustamycin and Tetrabid-Organon) and triple tetracycline (Deteclo) have no advantage over low-dose oxytetracycline (250 mg twice daily) in the treatment of NSU, but that minocycline (Minocin) offers a better cure rate. This effect was manifest by a lower relapse rate during the month after treatment (Table 2), and may be attributable to its greater lipophilicity facilitating higher tissue levels of the antibiotic (Macdonald *et al.*, 1973). However, it did not produce a lower relapse rate than low-dose oxytetracycline in the first trial. Thus, there seems to be a limiting cure rate of about 85%, which no antibiotic regimen can better, and it has been

suggested previously that psychological factors predetermine this phenomenon.

By extending the period of review from three to six months the number of patients who relapsed increased by 13%. Multiple relapses occurred among those patients who relapsed earliest (Figure) but all patients had been cured by the end of six months. During this time two patients were each treated for six relapses. Initial treatment proved the most successful with a relapse rate of 23% during the first two months, when 93% of those patients who relapsed repeatedly required further treatment. Subsequent treatment with a variety of regimens showed failure rates of between 36% and 63% (Table 3), twice that of initial treatment. Moreover, these patients received courses of treatment for up to three weeks. While this confirms the clinical impression that failure of first treatment carries an unfavourable prognosis, it also shows that the condition ultimately resolves. The findings also fail to support the view that some patients have an inherent susceptibility to NSU, since those with a past history were proportionately distributed between cured and relapsed groups.

Analysis of empirical treatment of contacts confirmed the previously reported conclusion (Evans, 1977) that neither partner appeared to benefit (Table 5), and that the relapse rate was as high among patients whose contacts were treated as among those whose contacts were not. It is conceivable that failure was caused by the contact not complying with the treatment and thus remaining 'infected' with a causal agent of NSU. Scrutiny of various clinical and laboratory findings may therefore reveal some difference in these contacts compared with those of patients who did not relapse, since this group would include contacts who were 'uninfected' before treatment. Table 6 shows that the groups did not differ in any marked way.

Findings among the 66 female contacts of patients with NSU for whom follow-up was satisfactory show some interesting results in a group little studied in the past. Symptoms were present in 29% of those in whom no other infection (that is, candidal, trichomonal, or gonococcal) was detected. The symptoms comprised vaginal discharge, pruritus vulvae, and dysuria, but mostly of mild degree, as might be anticipated in patients who did not present spontaneously but had been requested to attend for examination. It is difficult to draw conclusions from this finding in the absence of a control group similarly summoned for examination but in the proved absence of any disease in their sexual contacts. The abnormal appearance of the cervix noted in 44% was described as either 'erosion' or cervicitis, terms which vary widely in their

application, and this is also impossible to interpret without a control group. However a more recent sample of patients who were contacts of NSU was compared with a sample who had neither past nor present infection. This showed that an abnormal cervical appearance was found in 50% of the NSU contact group and in 56% of the control group (unpublished observations). Cervical secretions containing 10-20 pus cells per field might be considered within the range of normal variability in young sexually active women, 76% of whom are either taking an oral contraceptive or fitted with an intrauterine device. Furthermore, only 11% of the 95% of NSU contacts tested showed inflammatory changes in the epithelial cells on routine cervical cytological smear; 89% proved to have normal smears. Therefore, neither of these methods seems of any value in assessing the presence of infection in NSU contacts. Previous work has shown that 76% of contacts of gonorrhoea attending for examination are, in fact, infected (Evans, 1976) so that a figure of only 11% seems improbable in contacts of NSU.

The insuperable failure rate of NSU treatment may well be determined by a number of essentially psychological factors. Preoccupation with a previously ignored but sexually significant site, leading to excessive self-examination and therefore continual physical irritation, is well known to venereologists; these patients are sometimes referred to as 'squeezeers'. That this phenomenon should occur in NSU but not in gonorrhoea can be explained by the more gradual onset and slower response to treatment characteristic of NSU, leaving the patient with a longer period in which to develop the habit. Disregard of other aggravating factors such as continued sexual intercourse and alcohol consumption may also lead to relapse.

One explanation for these findings is that the man may acquire sufficient local immunity after adequate treatment of his initial infection to be able to resist reinfection for several weeks, during which time the infecting agent can be eliminated spontaneously by the woman in whom it appears to be of low pathogenicity. Thus, early relapses are caused by inadequate resolution of the original urethritis and later relapses by reinfection. This is supported by the isolation of *Chlamydia trachomatis* only in patients in whom relapse occurred more than six weeks after treatment (Handsfield *et al.*, 1976), and by the finding that only 15% of patients who relapsed within four weeks of treatment gave a history of re-exposure (Evans, 1977). It is also consistent with the present finding that minocycline reduces the incidence of early relapse but not the incidence of late relapse, since it would not be expected to protect against reinfection.

I should like to thank Mr James Bennett, SRN, RMN and his colleagues for invaluable help in the conduct of the trial, and the Department of Medical Illustration, Charing Cross Hospital, for the Figure.

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Postgonococcal urethritis

Relationship to penicillin sensitivity of the gonococcus

G. R. KINGHORN

From the Special Clinic, Royal Infirmary, Sheffield

SUMMARY Postgonococcal urethritis (PGU) occurred in 69 (21.3%) of 344 men with urethral gonorrhoea. Each case of gonorrhoea was treated with intramuscular procaine penicillin 2.4 megaunits and the penicillin sensitivity was determined. All 69 of the isolates from patients with PGU were fully sensitive to penicillin ($IC_{50} < 0.08$ iu penicillin per ml). Twenty-eight (10.2%) of 275 cases of gonorrhoea which were not followed by PGU were relatively resistant to penicillin. The persistence of gonococcal L-forms is therefore not likely to be a main cause of PGU. An incidental finding was that significantly more of the 69 men who developed PGU had a history of non-specific urethritis compared with the 224 men who fully recovered ($P < 0.01$).

Introduction

In a study that described a direct immunofluorescent test for identifying gonococcal L-forms, the possibility that these organisms were responsible for postgonococcal urethritis (PGU) was raised (Waitkins and Geary, 1977). L-forms may be induced by relatively low concentrations of penicillin (Lawson and Douglas, 1973) and their presence in urethral secretions of those suffering from gonorrhoea has been reported (Gnarpe *et al.*, 1972). The higher incidence of PGU after treatment with penicillin, compared with tetracycline (which does not induce L-forms), favours this hypothesis (Holmes *et al.*, 1967). Holmes *et al.* (1967) studied 49 patients treated with penicillin; the penicillin sensitivity was determined and PGU was more common after gonococcal infection caused by less sensitive organisms.

In the present study a retrospective analysis was made of the incidence of PGU related to the penicillin sensitivity of the gonococcus. This study was on a much larger group of patients, all of whom received intramuscular procaine penicillin 2.4 megaunits for urethral gonorrhoea. A statistical analysis of the results is given.

Patients and methods

The study period was from 1 May to 31 December 1976. Three hundred and forty-four consecutive

cases of urethral gonorrhoea, in 293 individuals, in whom the penicillin sensitivity of the causative gonococcus had been determined, were analysed. The initial treatment of each infection was intramuscular aqueous procaine penicillin 2.4 megaunits. The diagnosis of urethral gonorrhoea was made if Gram-negative diplococci were found on a stained smear of urethral discharge or scraping and if it was confirmed by positive results to culture of the organism, including sugar fermentation and/or fluorescent antibody tests.

The penicillin sensitivity of subcultures was determined using a plate dilution method (Reyn *et al.*, 1963). The results obtained were expressed as 50% inhibitory concentration (IC_{50}), and were standardised using reference strains from the State Serum Institute, Copenhagen. Cultures with IC_{50} greater than 0.08 iu penicillin per ml were regarded as relatively resistant.

If gonococci still persisted in the urethral smear or on urethral culture within one week of treatment, the treatment was regarded as having failed. The diagnosis of PGU was made in those who had a persistent purulent or mucopurulent urethral discharge seven days after treatment of the initial infection, or in whom a discharge reappeared within 28 days of primary treatment. Specimens of urethral secretion were taken with a wire loop after urethral massage. Gram-stained smears and a wet film for trichomonads were prepared. The finding of eight or more leucocytes per field using a $\times 100$ oil immersion objective together with negative findings for gonococci and trichomonads by smear and culture, was considered diagnostic of non-specific

Address for reprints: Dr G. R. Kinghorn, Special Clinic, Royal Infirmary, Sheffield S6 3DA

Received for publication 10 October 1977

urethritis. In addition all patients had pus in the urine manifest as haze, flocculations, or threads in one or both glasses.

Yates's χ^2 test was used for statistical calculations.

Results

PGU was diagnosed in 69 (21.3%) of the 344 infections. These 69 men constituted the postgonococcal urethritis group (PGU Group) and were compared with 224 men who had no evidence of persisting urethral discharge—the gonorrhoea only group (GO Group). Thirty-seven (12.6%) men, all in the GO Group, had more than one gonococcal infection. Mean age, country of origin, and marital state were similar in both groups (Table 1). Fifteen (21.7%) of 69 patients in the PGU Group compared with 67 (29.9%) of 224 patients in the GO Group had had gonorrhoea previously. This difference was not significant ($P > 0.1$). Only a few patients in both groups had a history of trichomoniasis. There was,

however, a significant difference in the incidence of patients with a history of non-specific urethritis ($P < 0.01$). Such a history was present in 20 (29%) of 69 patients in the PGU Group, but only in 30 (13.4%) of 224 patients in the GO Group.

The gonococci isolated in all 69 infections in the PGU Group showed an IC_{50} less than 0.08 iu of penicillin per ml—that is, they were sensitive strains. In the GO Group 28 (10.2%) of 275 infections were caused by organisms which were relatively resistant to penicillin; all organisms showed an IC_{50} greater than 0.1 iu penicillin per ml. Two hundred and forty-seven (89.8%) of the 275 strains were sensitive. The difference between the two groups was significant ($P < 0.02$), Table 2.

There were six treatment failures in the GO Group owing to relatively resistant organisms. Secondary treatment of three patients with kanamycin 2 g intramuscularly and three with spectinomycin 2 g intramuscularly was successful. After initial treatment with penicillin no treatment failure was diagnosed in any infection due to a sensitive organism in either group.

Table 1 History and details of patients

Postgonococcal urethritis (no. of infections 69; no. of individuals 69)		Gonococcal urethritis only (no. of infections 275; no. of individuals 224)	
No.	%	No.	%
Age groups			
Younger than 16 years	—	1	0.4
16-19	18	38	17.0
20-24	26	71	31.7
25-34	20	85	37.9
35-44	3	20	8.9
45 and over	2	9	3.8
Mean $\pm$ SD	24.82 $\pm$ 6.88	26.54 $\pm$ 7.40	
Country of origin			
UK	49	168	75.0
West Indies	14	36	16.1
Europe	—	4	1.8
Africa	—	4	1.8
Middle East	5	5	2.2
Pakistan	1	6	2.7
South America	—	1	0.4
Marital state			
Single	48	145	64.7
Married	15	56	25.0
Separated	3	13	5.8
Divorced	3	10	4.5
History			
Gonorrhoea	15	67	29.9
Non-specific genital infection	20	30	13.4
Trichomoniasis	2	6	2.7

*Difference by Yates's χ^2 test $\chi^2 = 1.37$

At one degree of freedom $P > 0.1$

†Difference by Yates's χ^2 test $\chi^2 = 7.99$

At one degree of freedom $0.01 > P > 0.005$

Table 2 Sensitivity of strains

	PGU group		GO group	
	No.	(%)	No.	(%)
No. of infections in which IC_{50} was determined	69		275	
$IC_{50} > 0.08$ iu penicillin per ml	—		28	(10.2)
$IC_{50} < 0.08$ iu penicillin per ml	69	(100)	247	(89.8)

$\chi^2 = 6.35$ (using Yates's modification)

At one degree of freedom $0.02 > P > 0.01$

Discussion

If gonococcal L-forms, whose formation may be induced by relatively low penicillin concentrations, are a main cause of PGU, it would be expected that a greater proportion of infections caused by gonococci that are relatively resistant to penicillin would be followed by this condition as reported by Holmes *et al.* (1967). The results reported here contrast with those obtained by Holmes *et al.* (1967). An unexpected finding was that all cases of PGU followed gonococcal infections that were fully sensitive to penicillin. If an infective agent is the cause of PGU it is likely to be other than the gonococcal L-form. The PGU Group and GO Group differed in the proportion of patients with a history of non-specific urethritis. Other differences that were not assessed but which may be important are contraceptive methods, alcohol intake, and sexual behaviour.

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Current approaches to the diagnosis of herpes genitalis

E. M. BELSEY AND M. W. ADLER

From the Departments of Venereology and Genitourinary Medicine and Community Medicine, Middlesex Hospital and Medical School, London

SUMMARY The current methods of diagnosing and treating herpes genitalis in clinics for sexually transmitted diseases in England and Wales are reported. Virus cultures were used as a diagnostic test in some or all patients in nearly two-thirds of clinics. Darkground examinations were performed on all or selected patients in most clinics, but fewer than half the clinics performed them on three or more separate occasions. In over 80% of clinics attempts were made to see some or all contacts of patients with herpes. The treatments prescribed varied, the commonest being saline washes, idoxuridine, and oral antibiotics. Serological tests were repeated after the disappearance of the lesions in 90% of clinics, while in 60% of clinics women with herpes genitalis were advised to have cytological examination at regular intervals in future. The importance of excluding syphilis in patients presenting with genital ulceration, the most appropriate treatments for herpes, and the possible association between the disease and cervical carcinoma are discussed.

Introduction

Although herpes genitalis remains one of the less common sexually transmitted diseases seen in clinics, it does present several special problems. Firstly, the disease often recurs; secondly, an effective treatment has yet to be found; thirdly, the question of its association with cervical cancer remains unresolved. The importance of herpes genitalis should therefore not be underestimated.

The methods used by clinics for sexually transmitted diseases in England and Wales were surveyed (Adler *et al.*, 1978). The diagnostic techniques used for herpes genitalis and its treatment are now reported.

Results

DIAGNOSTIC FACILITIES AND THEIR USE

Cultures

In a previous paper (Adler *et al.*, 1978) it was reported that, of the 175 clinics in England and Wales included in the survey, 171 treated men and 173 treated women. In 126 (74%) of the clinics where men were treated and in 127 (73%) where women were seen a virus culture service was available. However, it was not used in 18 (14%) of the clinics for men nor in the same number of clinics for women, even

though it was available. In men presenting with genital ulceration, virus cultures were used routinely to make or exclude the diagnosis of herpes genitalis in 55% of clinics, and in women this was so in 56% of clinics (Table 1). In a further 8% of clinics virus cultures were used for selected male patients, while in 7% they were used for selected females. In more than one-third of clinics, however, the diagnosis was made from the clinical history and examination, without pathological confirmation.

In clinics where virus cultures were not used in every case the most common reasons for selecting patients were that the attack of herpes was particularly severe, or that the doctor was unable to determine clinically whether the patient was suffering from herpes or from syphilis.

Serum antibody complement-fixation tests and cytology

Serum antibody complement-fixation tests were performed in 30% of all clinics. Routine cytology

Table 1 *Methods used to diagnose herpes genitalis in addition to clinical history and examination*

Method	Men		Women	
	No.	%	No.	%
Virus cultures				
In all cases	94	55.0	97	56.1
In selected cases	14	8.2	12	6.9
No pathological confirmation	63	36.8	64	37.0
	171	100.0	173	100.0

Address for reprints: E. M. Belsey, Medical Research Council, Sexually Transmitted Diseases Project, James Pringle House, 73-75 Charlotte Street, London W1P 1LB

Received for publication 11 October 1977

was carried out in over half (54%) of the clinics treating women, but cervical virus cultures were performed in only 44 clinics, 35% of those with a virus culture service.

Darkground microscopy

Darkground examinations to exclude syphilis were performed on all men with genital ulceration in 58% of clinics, on selected patients in 36%, and were not performed at all in 11 clinics (6%) (Table 2). The corresponding figures for women were similar. Table 3 shows the reasons given for selecting

Table 2 Clinics performing darkground examinations on patients with genital ulceration

Darkground examination	Men		Women	
	No.	%	No.	%
All patients	99	57.9	96	55.5
Selected patients	61	35.7	66	38.1
Never	11	6.4	11	6.4
	171	100.0	173	100.0

Table 3 Reasons given for selecting cases for darkground examination

Reason	Men (N=61)		Women (N=66)	
	No.	%	No.	%
Clinical appearance of syphilis/atypical of herpes	57	93.4	62	93.9
Patient's occupation	5	8.2	5	7.6
Homosexual patient	4	6.6	0	0.0
Other	6	9.8	6	9.1

patients for darkground examinations if they were not performed routinely. The criterion for selection most often given, by consultants working in 57 clinics for men and 62 clinics for women, was that the case was atypical of herpes or had the clinical appearance of syphilis. Other reasons given were that the patient was homosexual or had an occupation likely to lead to a relatively high risk of contracting syphilis. In 51 clinics for men only one of the reasons listed was mentioned, while two reasons were given in nine, and three in one clinic; in 59 clinics for women one reason was mentioned and two reasons were given in seven clinics.

Although a large proportion of physicians reported that darkground examinations were performed to establish the initial diagnosis in some or all cases, in 30% of the clinics they were carried out once only (Table 4). In a further 30% of clinics they were performed on two occasions, while in the remaining clinics they were performed three or more times. The number of occasions on which darkground examinations were carried out did not differ

between clinics in which they were performed in all cases and those in which they were performed in selected cases.

FREQUENCY OF DIAGNOSTIC TESTS

Clinics in which virus cultures were used routinely as a method of diagnosis were compared with those which used them in selected cases and those which did not use them at all, to find out if these figures differed from those on darkground examinations to exclude syphilis. It was found that darkground examinations were equally likely to be performed in clinics using virus cultures in some or all cases as in those clinics in which virus cultures were not used. The number of darkground examinations performed was also found not to differ between clinics using cultures in all cases, in selected cases, or not at all.

EXAMINATION OF CONTACTS

In approximately one-half of clinics attempts were made to see all contacts of patients suffering from herpes genitalis (Table 5). Consultants working in another 32% of clinics tried to see contacts in selected cases, while in the remaining clinics no attempts were made. Table 6 shows the criteria for

Table 4 Number of occasions on which darkground examinations performed

No. of occasions	Men		Women	
	No.	%	No.	%
One	48	30.0	49	30.2
Two	49	30.6	49	30.2
Three	58	36.3	59	36.4
More than three	5	3.1	5	3.1
	160	100.0	162	100.0

Table 5 Clinics attempting to see contacts of patients with herpes genitalis

Contacts	Male contacts		Female contacts	
	No.	%	No.	%
All	84	49.1	85	50.3
Selected	54	31.6	55	32.5
None	33	19.3	29	17.2
	171*	100.0	169	100.0

*169 clinics seeing both sexes, plus two clinics seeing only men

Table 6 Reasons given for seeing selected contacts

Reason	Male contacts (N=54)		Female contacts (N=55)	
	No.	%	No.	%
Symptoms/positive history	31	57.4	30	54.5
Regular partner	13	24.1	16	29.1
Recurrent attacks in original patient	11	20.4	11	20.0
If severe in female	2	3.7	0	0.0
Not known	0	0.0	3	5.5

seeing contacts given by the clinics in which only selected contacts were seen. The most common reason was that the patient reported that his or her sexual contact had signs or symptoms of genital ulceration. Some consultants were even more selective and would see only the patient's regular partner or contacts of patients suffering from recurrent attacks of herpes. In 51 clinics only one criterion for seeing male contacts was given, while in three clinics two criteria were mentioned; of the clinics in which attempts to see selected female contacts were made, one criterion was given in 47, two were given in five, and in three clinics the criteria used for selection were not ascertained.

TREATMENT OF PATIENTS

Male and female patients with lesions suggestive of herpes were treated before the results of cultures or serological tests for syphilis were known in 90% and 91% of clinics, respectively (Table 7). In 12 (7%) clinics consultants reported that they would treat selected male patients and in nine (5%) that they would treat selected females immediately, if, for example, they were certain clinically that the disease was herpes or if the patient was in severe pain. In a few clinics patients were treated only after the results of cultures and/or serological tests for syphilis had been obtained.

The treatments varied between clinics. Table 8 shows primary and secondary courses of treatment for both men and women. Primary treatment is defined as that which would be given routinely at the time the diagnosis of herpes was made in cases where the attack was not unusually severe; secondary

Table 7 Clinics treating before results of cultures or serological tests for syphilis

Treatment before results	Men		Women	
	No.	%	No.	%
Patients				
All	154	90.1	157	90.8
Selected	12	7.0	9	5.2
None	5	2.9	7	4.0
	171	100.0	173	100.0

treatment is that which would be used in the first instance only if the attack of herpes was severe, or would be used at a later stage if the lesions had become infected. In almost half the clinics saline washes were initially recommended. Idoxuridine, in strengths ranging from 0.5% to 40%, was prescribed as the primary treatment for men and women in 22% and 23% of clinics, respectively. In an additional 12% and 14% of clinics, consultants reported that they would prescribe idoxuridine as a secondary form of treatment. Oral antibiotics were given to all patients in the first instance in approximately one-quarter of clinics, and in almost the same number again, antibiotics would be given if the lesions became infected.

Some form of topical preparation other than idoxuridine was prescribed in approximately 40% of clinics, usually as a primary course of treatment. These included antibiotic and corticosteroid creams, various spirits, gentian violet, potassium permanganate, and dusting powder. Interferon was used in two clinics, photodynamic inactivation in one, and in three clinics amantadine hydrochloride was prescribed for both men and women.

Table 8 Treatments prescribed for herpes genitalis

Treatment	Primary				Secondary			
	Men (N=171)		Women (N=173)		Men (N=171)		Women (N=173)	
	No.	%	No.	%	No.	%	No.	%
None	1	0.6	6	3.5	0	0.0	5	2.9
Saline washes	82	48.0	82	47.4	0	0.0	0	0.0
Idoxuridine	38	22.2	39	22.5	21	12.3	24	13.9
Oral antibiotics	42	24.6	46	26.6	37	21.6	41	23.7
Analgesics	7	4.1	7	4.0	1	0.6	1	0.6
Topical preparations	65	38.0	64	37.0	4	2.3	5	2.9
Antibiotic	9		8		0		0	
Corticosteroid	4		3		3		3	
Antibiotic with corticosteroid	0		2		0		0	
Spirits (ether, methylated, surgical)	8		6		0		0	
Gentian violet/copper sulphate/potassium permanganate	10		13		0		0	
Analgesic creams	3		4		1		2	
Dusting powder	14		15		0		0	
Other	17		13		0		0	
Interferon	2	1.2	2	1.2	0	0.0	0	0.0
Amantadine hydrochlor (Symmetrel)	3	1.8	3	1.7	0	0.0	0	0.0
Photodynamic inactivation	0	0.0	1	0.6	1	0.6	1	0.6

FOLLOW-UP TESTS

Serological tests

Table 9 shows that in a large proportion of clinics serological tests for syphilis were repeated at least once after the disappearance of the lesions. However, the last test was carried out less than three months after the patient's initial exposure in 38 (25%) of the 154 clinics repeating tests. There was no difference between the clinics that used cultures routinely, in selected cases or not at all, with regard to whether or not serological tests were repeated, the number of repeat tests, or the interval between exposure and final test.

Table 9 *Number of occasions on which serological tests for syphilis performed after disappearance of ulceration*

No. of occasions	Men		Women	
	No.	%	No.	%
None	17	9.9	19	11.0
One	78	45.6	76	43.9
Two	46	26.9	46	26.6
Three	21	12.3	22	12.7
More than three	7	4.1	8	4.6
Not known	2	1.2	2	1.2
	171	100.0	173	100.0

Cervical cytology

Women with herpes genitalis were advised to have regular cytological examination, usually at yearly intervals, in 104 (60%) of the 173 clinics.

QUARTERLY RETURNS FOR HERPES GENITALIS

In all clinics where virus cultures were used, it was reported that cases with a positive virus culture would be included in the quarterly returns for herpes genitalis to the Department of Health and Social Security (DHSS). In 77 of the 171 clinics for men, all or selected patients were treated for herpes genitalis on clinical evidence as virus cultures were not used. The consultants working in 75 (97%) of these clinics reported that they would include such cases in the numbers reported to the DHSS. Similarly, in 75 (99%) of the 76 clinics in which all or some female patients were treated without pathological confirmation of the diagnosis, it was stated that these cases would be included in the quarterly returns for herpes genitalis.

Discussion

DIAGNOSTIC TESTS

In a large proportion (94%) of clinics darkground examinations to exclude syphilis were performed in some or all patients presenting with genital ulceration. However, such examinations should be per-

formed on at least three separate occasions to ensure a good chance of identifying *Treponema pallidum* if it is present (Catterall, 1974; Schofield, 1975). Darkground examinations were performed as often as this in fewer than 40% of clinics, while in 30% only one examination was performed. The same principle applies to serological tests for syphilis. These should be repeated three months after the patient's initial exposure. This survey has shown that although in approximately 90% of clinics serological tests were repeated after the disappearance of the ulceration in both men and women, in one-quarter of these the last test was performed less than three months after the patient's initial exposure to risk.

TREATMENT

There is no universally accepted way of treating herpes genitalis, and the diversity of treatments reflects the inadequacy of current treatment. Virtually all the topical preparations recommended or prescribed—such as saline washes, spirits, paints, and dusting powders—can do little more than give a limited amount of symptomatic relief.

Idoxuridine (IDU) was used as a primary or secondary form of treatment in more than one-third of clinics. While there have been many trials to determine the potential of IDU in shortening the duration of attacks of herpes or preventing recurrences, the reports have been contradictory. Hutfield (1964) found that 0.5% IDU in ointment shortened the duration of genital herpetic lesions after the beginning of treatment by 2.8 days, compared with an inert preparation. Five per cent IDU in dimethylsulphoxide (DMSO) was shown by MacCallum and Juel-Jensen (1966) to shorten the duration of attacks of oral herpes by 6.3 days, but DMSO alone also resulted in a decrease of 4.1 days. Schofield (1964) reported that of 54 patients treated with 0.5% IDU in liquid paraffin and white soft paraffin on both oral and genital lesions, only three reported recurrences, but his low follow-up rate must cast doubt upon this finding. Four weeks after their initial visit, over 50% of the original group had been lost to follow-up, and by three months this proportion had reached 88%.

In contrast, Ng *et al.* (1970) reported that they found IDU to have no beneficial effect on the duration of the infection or on the prevention of recurrent disease. In a study comparing photodynamic inactivation, 0.5% IDU ointment, and normal saline there was no difference between IDU and saline with respect to the relief of pain, the healing time, or the time taken for the virus to disappear as measured by cultures (Taylor and Doherty, 1975). A recent double-blind controlled

trial comparing 40% IDU in DMSO with DMSO alone also showed no evidence that the addition of IDU improved the results of treatment in any way (Morgan, 1977, personal communication).

Even if IDU were proved to be beneficial, its side effects—such as contact dermatitis—would militate against its widespread use. Furthermore, Green and Staal (1976) warned against the indiscriminate use of topical IDU in strengths of 5% or greater, because of its mutagenic potential. In cells pretreated with IDU, they and other workers have found enhanced susceptibility of resistant cell types to infection by cytomegalovirus and adenoviruses, enhanced replication of many other RNA and DNA viruses, and induction of infectious virus production in cells with integrated viral genomes, including viruses associated with human and animal neoplasia.

We have reported that photodynamic inactivation has not been widely adopted as a method of treatment for herpes genitalis in this country. An early report of its effects was encouraging (Felber *et al.*, 1973) but two subsequent studies in which neutral red dye was compared with a non-photoactive dye, phenolsulfonphthalein, failed to show any difference in the duration of patients' discomfort, the healing time of the lesions, the time taken to eliminate the infective virus, or the length of time between recurrences (Myers *et al.*, 1975; Roome *et al.*, 1975). Cusumano and Monif (1975) also suggested that the inactivation of the virus is only partial. While the damage is sufficient to prevent it from producing lesions, the apparently inactivated virus particles can still be capable of transforming a cell, and thus giving rise to local malignancies, but only after a delay of many years.

It is understandable that many consultants, although aware that little can be done to shorten the duration of the lesions, hope that some form of therapy will be of some psychological benefit to the patient. However, in view of the equivocal evidence on the efficacy of IDU and photodynamic inactivation, their potential side effects and expense, it is suggested that their use is not justified.

CARCINOMA OF THE CERVIX

The possibility of an association between herpes genitalis and carcinoma of the cervix has now been the subject of debate for more than a decade. Evidence supporting the existence of a relationship has been mainly provided by a series of sero-epidemiological studies comparing the prevalence of antibodies to herpes virus Type 2 among women with dysplasia, carcinoma *in situ*, and invasive carcinoma with that found in control groups (Rawls *et al.*, 1968, 1969; Nahmias *et al.*, 1970; Royston and Aurelian, 1970; Catalano and Johnson,

1971; Skinner *et al.*, 1971). These studies consistently show that positive antibodies are more common among women with dysplasia, carcinoma *in situ*, and invasive carcinoma than among their respective controls, and furthermore that they are less common among women with dysplasia than among those with carcinoma *in situ*, and most prevalent among women with invasive carcinoma. However, the control groups in these studies were not matched for age at first sexual intercourse or number of sexual partners. Since both of these factors have been shown to be related to the prevalence of carcinoma of the cervix (Lombard and Potter, 1950; Wynder *et al.*, 1954), it is possible that sexual promiscuity is a covariable affecting both the risk of developing cervical cancer and the risk of exposure to herpes genitalis.

More recently, Nahmias *et al.* (1973) reported preliminary results of a prospective study undertaken to evaluate the risk of cervical anaplasia developing after the detection of genital herpetic infection. They found that the rate of cervical dysplasia was twice as great, and that of *in situ* carcinoma eight times higher in the herpes group than in a control group in whom Type 2 antibodies could not be detected.

Despite these reports, it still cannot be said that herpes genitalis causes cervical cancer. However, the virus is under sufficient suspicion for it to be strongly recommended that a viral culture service should be available to all clinics and that cervical virus cultures should be performed routinely. Furthermore, all women with herpes genitalis should be advised to have cytological examination at regular intervals. This study has shown that cervical virus cultures were performed in only one-third of clinics with a virus culture service, and that in 40% of clinics women with herpes genitalis were not recommended to have cytology.

Conclusions

It is important that syphilis be excluded in all patients with genital ulceration. It is to be hoped, therefore, that consultants will increase both the number of patients on whom darkground examinations are performed and the number of occasions on which these examinations are carried out, and that final serological tests for syphilis be repeated at least three months after initial exposure to risk. In view of the relative ineffectiveness of IDU and photodynamic inactivation it is suggested that palliative therapy is the most appropriate and least expensive treatment. The association between cervical carcinoma and herpes genitalis is as yet not proved but the evidence is such as to warrant careful

cytological follow-up of all women with genital herpes.

This study was supported as part of a project grant from the Medical Research Council. We should like to thank Dr B. H. O'Connor for carrying out a substantial number of interviews. We are most grateful to Dr R. D. Catterall and Professor D. L. Miller for their encouragement and support and to Dr C. S. Nicol and Dr R. S. Morton for their advice. We are particularly grateful to Dr R. R. Willcox for his generous advice on the design of the study.

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Acquisition of Type 1 herpes simplex vulvitis within a monogamous relationship

JANET E. WHITNEY, G. R. B. SKINNER, AND A. BUCHAN

From the Department of Medical Microbiology, The Medical School, Birmingham

Although Type 1 herpes simplex virus infections of the genitals have been reported (Smith *et al.*, 1973; Chang *et al.*, 1974; Dolin *et al.*, 1976) in women, Type 2 herpes virus is more commonly isolated; in extensive surveys by Dowdle *et al.* (1967), Plummer *et al.* (1974), and Skinner *et al.* (1976) every isolate from the female genital tract was found to be Type 2. Recognition of the sexual mode of transmission of this condition preceded the 'discovery' of viruses; Greenough (1881) correlated the incidence of the condition in men with a history of other venereal disorders, and Unna (1883) reported that 1% of prostitutes attending the Hamburg General Hospital had genital herpes. More recently, the sexual transmission of this infection was confirmed by virological follow-up of female consorts of male patients with penile herpes (Nahmias *et al.*, 1969).

In this paper, we report a case of Type 1 herpes virus infection of the vulva which almost certainly was contracted by orogenital contact between marital partners.

Case report

The patient was a 20-year-old laboratory technician. She had been married for 11 months with no pregnancies or abortions. Neither the patient nor her husband reported pre-marital or extramarital sexual relations.

Forty-eight hours after oral sex with her husband, the patient became aware of a tender area on the vulva. There was no vaginal discharge although the patient did complain of superficial dysuria which was not accompanied by frequency or urgency of micturition. There was no upset or irregularity of menstrual function. She did not give a history of recurrent cold sores on the lips.

The patient's husband reported that he had a cold sore on his lips 48 hours before the episode of

oral sex. There was no evidence of digital or penile herpes infection.

Examination revealed three tender ulcers around the clitoris. There were no other genital abnormalities.

A presumptive clinical diagnosis of herpetic vulvitis was made and the appropriate laboratory specimens for virological investigation were taken. The patient was advised to avoid touching the lesions and to abstain from sexual intercourse. Iodoxuridine (5 g/100 ml of dimethylsulphoxide-DMSO), was applied to the lesions and the patient asked to continue treatment thrice daily for three days. Within five to six days the lesions had almost resolved and the tenderness had disappeared by the eighth day from onset.

VIROLOGICAL INVESTIGATIONS

Herpes simplex virus was isolated in BHK-21 cells (MacPherson and Stoker, 1962) as previously described (Skinner, 1976). The virus was shown to be Type 1 herpes simplex virus by its behaviour in reciprocal cross-neutralisation and immunodiffusion tests with Type 1 and Type 2 antiserum raised in rabbits (Thouless, 1972; Skinner *et al.*, 1976), and by the stability of its enzyme—thymidine kinase (Thouless and Skinner, 1971) (Table 1). The validity of these various criteria for virus typing has been discussed (Plummer *et al.*, 1974). Unfortunately, we were unable to isolate virus from the healing lesions on the husband's lips, probably because he was seen too late in the course of his infection.

Serum complement-fixing antibody activity against both types of viral antigen was found within three weeks from the onset of the lesions and reached a peak within about seven weeks of the original lesion. There was, however, demonstrable complement-fixing activity still present within one year of the clinical lesion (Table 2). There was an unusually low serum neutralising antibody activity against both virus types for 10 months after the appearance of the clinical lesion when the serum

Address for reprints: G. R. B. Skinner, Department of Medical Microbiology, The Medical School, Birmingham B15 2TJ

Received for publication 12 August 1977

Table 1 *Evidence that clinical isolate was a Type 1 herpes simplex virus*

	Neutralisation† rate constants (k-value)			Immunodiffusion pattern	Stability of‡ thymidine kinase
	Type 1 antisera	Type 2 antisera	Ratio Type 1:2		
Isolate from patient	3.4	1.8	1.8	Type 1	85
Prototypes					
Type 1 strain 'HFEM'*	3.1	2.0	1.6	Type 1	92
Type 2 strain '25766'*	0.5	1.5	0.3	Type 2	40

*The provenance and prototype of these strains has been previously established (Skinner *et al.*, 1976)

†K-values were obtained and calculated as previously described (Skinner *et al.*, 1976)

‡Percentage residual thymidine kinase activity after 15 min at 40°C

specimen was found to have a Type 1 k-value of 0.58 and a Type 2 value of 0.22, which was consistent with previous infection by Type 1 herpes simplex. The patient's cervical cytology was negative at all times.

The serum of the patient's husband had a neutralising antibody activity of 1.8 against Type 1 and 0.33 against Type 2. This also suggested a history of Type 1 virus infection.

The patient was followed-up at regular intervals for a year. There was no further evidence of clinical disease, herpes virus was not isolated, and the cervical cytology remained negative.

Fifteen months after the primary infection, the patient reported a further episode of vulval tenderness. Extramarital sexual relations had not occurred and, while normal marital sexual intercourse had taken place once or twice a week, the last orogenital contact had been several months before. The patient did report being rather 'run-down' after a recent severe bout of influenza.

Examination revealed four typical herpetic lesions around the clitoris. The lesions were smaller than during the previous clinical episode—about 2 to 4 mm—and seemed rather less tender to palpation. No other gynaecological abnormality was apparent.

The patient was treated as before (*vide supra*). On this occasion the lesions completely disappeared four days after their onset.

Type 1 herpes simplex virus was again isolated.

The serum neutralising and complement-fixing antibody activity rose (Table 2), again suggesting previous Type 1 herpes virus infection. The latest specimen, obtained 10 days after recurrence of the clinical lesion (Table 2), demonstrated a common immunoprecipitating antibody between Type 1 and Type 2 herpes simplex virus.

Discussion

A case of primary and recurrent Type 1 herpetic vulvitis has been described. Two important features emerge:

The history of oral sex with a male partner suffering from a labial herpetic lesion, together with the unequivocal laboratory evidence that the aetiological agent at primary presentation and at recurrence was herpes simplex virus Type 1 indicates that herpetic vulvitis is not an exclusively genital-to-genital 'venereal disease' and can occur within the framework of a monogamous relationship. The case is not, of course, proved but it might discourage excess contact inquiries in other cases. The value of virus isolation and typing is apparent.

A second point of interest concerns the feeble delayed primary neutralising antibody response in this patient. We also observed this phenomenon on two other patients who had exclusively introital lesions which were of limited extent and duration,

Table 2 *Serological reactivity from date of primary infection*

Date	Neutralisation rate constants (k-value)		Reciprocal complement-fixation titre against viral antigen	
	Type 1 virus	Type 2 virus	Type 1	Type 2
12.12.75	Primary episode of herpetic vulvitis		<25	<25
15.12.76	0.0	0.0	50	<25
5.1.76	0.02	0.0	200	<50
12.2.76	0.07	0.06	100	<50
7.7.76	0.06	0.04	100	50
27.10.76	0.58	0.22	<50	<50
6.3.77	Recurrence of herpetic vulvitis			
10.3.77	1.14	0.15	200	50
16.3.77	1.02	0.17	100	50

K-values and complement-fixing titres were obtained and calculated as previously described (Skinner *et al.*, 1976).

Complement-fixing titres of sera 12.12.75 and 15.12.75 were obtained with different antigen preparations than were used with the other sera.

and it may be that the feeble neutralising antibody response is related to an inadequate dose of priming antigen. This is consistent with the observations of Birch *et al.* (1976) who reported that Type 1 herpes virus grew less well and produced less antigen in cervical organ culture. The neutralising antibody activity did, however, significantly increase between July and October 1976 and must be presumed to be a result of a 'subclinical recurrence' of infection during that period of time.

Recurrence of herpes genitalis is a difficult therapeutic problem but seems to respond in some measure to 'persistent' idoxuridine therapy (Juel-Jensen and MacCallum, 1972). In our case, the absence of extramarital contact, the identical distribution of the herpetic lesions on recurrence and the reisolation of Type 1 herpes simplex virus from the lesions is strong evidence in favour of a recurrence from a latent virus infection rather than an exogenous reinfection. This poses future problems in management, particularly towards avoiding the establishment of penile herpes infection in the husband and, of course, in a possible fetus. On this account, the patient has been advised to report immediately any further symptoms and abstain from sexual intercourse until the lesions appear or until positive viral isolations have been made. In this event it is intended to resume idoxuridine treatment.

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Default patterns of patients attending clinics for sexually transmitted diseases

J. D. H. MAHONY, JOHN BEVAN, AND BARBARA WALL

From St Giles' Hospital, London, and the Health Services Research Unit, University of Kent at Canterbury

SUMMARY The influence of gender, propaganda, and treatment methods was studied in relation to default behaviour of patients with sexually transmitted diseases. The overall default rate of men and women was similar, but a larger proportion of men defaulted after the initial visit, while the biggest fall-out in women was after the second attendance at the clinic. The institution of a propaganda campaign was followed by a reduction in defaulting. The statistical significance of this is open to question, however: moreover the observed improvement in default rate was not maintained once the propaganda had been relaxed. Men treated for non-gonococcal urethritis by a regimen which included one injection a week for three weeks showed a highly significantly lower default rate compared with those who received tablets alone.

Introduction

The term 'defaulter' is well recognised in clinics dealing with sexually transmissible diseases. Although it is impossible to give a scientifically precise definition of this term, it has real meaning for venereologists and seems, despite its quasi-military associations, to be preferable to the term 'patient drop-out'. In a comprehensive review article, Blackwell (1976) uses the terms 'patient compliance' and 'patient adherence', but he uses them with special reference to treatment schedules rather than for routine follow-up attendances. He makes the point that the word 'compliance' has no counterpart in the German or Dutch languages. Venereologists are just as interested in 'treatment adherence' as any other doctor who prescribes treatment, but their primary aim must be to prevent default since a high default rate precludes an accurate estimation of either treatment adherence or therapeutic response. Hewitt (1969) devised an ingenious formula for assessing therapeutic response which took into account those patients who had defaulted after treatment for gonorrhoea, but it seems preferable to make a therapeutic estimate solely by assessing those patients who do not default. It is desirable to adopt any measures that reduce the default rate, since the lower this is, the more valid are the conclusions arising from any assessment of treatment.

Address for reprints: J. D. H. Mahony, MD, FRCP, Consultant Venereologist, St Giles' Hospital, St Giles' Road, London SE5 7RN
Received for publication 2 August 1977

Patients and methods

PATIENTS

Men v. women (Group A patients)

The first part of the study was carried out on all patients attending the sexually transmitted disease clinic at the Kent and Canterbury Hospital from 1 January to 30 June 1975. This comprised 224 men and 139 women. In this instance, a defaulter was defined as a patient who was judged to have failed, of his or her own volition, to complete the period of supervision, usually three months, recommended by the doctor at the first interview. (This rather imprecise definition is applicable only to Group A and Group C patients.) In the rest of the study the one- and two-attendance default rate—a more precisely measurable parameter—is considered.

Effect of propaganda and intensive counselling (Group B patients)

A comparative study was made on one- and two-attendance default rate of 53 men with gonorrhoea who attended the Alexandra Clinic at St Giles' Hospital in July 1975, and 45 men with gonorrhoea who attended the clinic in July 1976. Those attending in July 1976 were subjected to intensive exhortation and counselling by medical and nursing staff at their first visit. The importance of refraining under surveillance was stressed and verbal advice was reinforced with posters displayed in the waiting room and reception areas.

A parallel study was also carried out between men with non-gonococcal urethritis (NGU) who attended St Giles' Hospital in July 1975 and July 1976.

Exhortation and counselling were reduced to normal levels in August 1976 and the one- and two-attendance default rates of patients with gonorrhoea were again analysed.

Finally, the combined one- and two-attendance default rate for men with gonorrhoea, plus men with NGU for July 1976, was compared with that for July 1975.

Effect of treatment schedules (Group C patients)

These comprised 217 men who had taken part in a combined clinical trial between St Giles' Hospital and the Kent and Canterbury Hospital from January to December 1974.

Two treatment schedules for NGU had been compared for therapeutic efficiency and influence on default rate. One regimen consisted of three injections of streptomycin sulphate (1g intramuscularly) given at weekly intervals. This was combined with a simultaneous oral dose of 2 g of the ultra long-acting sulphonamide, sulfametypyrazine (Kelfizine). The other regimen consisted of administering an oral dose of one tablet of the triple tetracycline, Deteclo, twice a day for three weeks. These patients were asked to report weekly for the next two weeks and, thereafter, at prescribed intervals for a total of 12 weeks.

FACTORS THAT MAY INFLUENCE DEFAULT RATES

The factors with a possible influence on default rates considered in this study were:

- Gender of the patient (male or female)
- Propaganda and counselling
- Treatment methods

The effect of sending 'default letters' or reminders to patients who had not kept their appointments was also assessed for Group A patients.

Results

MEN V. WOMEN

Table 1 Overall default rate at Kent and Canterbury Hospital

Men	Women
122 —=54.5% 224	68 —=48.9% 139

Table 1 shows that there was little difference between the overall default rates of men and women in the

patients attending Kent and Canterbury Hospital (January to June 1975).

Table 2 Proportion of total defaulters after first two attendances at Kent and Canterbury Hospital

Defaulters	Men	Women
After one visit	41 —=33.6% 122	16 —=23.5% 68
After two visits	28 —=23.0% 122	27 —=39.7% 68
Others	53 —=43.4% 122	25 —=36.8% 68

$$\chi^2=6.2; P<0.05$$

The results suggest that one-third of men who default do so after the first attendance, whereas less than one-quarter of women who default cease to attend at this stage (Table 2). On the other hand, nearly 40% of women who default do so after the second attendance, whereas less than one-quarter of the male defaulters cease attending after this attendance. The total default rate after the first two visits is similar for both sexes and comprises more than half of the total default rate.

The response rate to 'default letters' sent to patients who had missed an appointment was poor for both sexes. A total of 93 default letters were sent to the 122 men and there was no response in 81 (87%) of these. An almost identical 'non-response rate' of 86% was obtained from the women. These results include a small proportion of patients of either sex who were sent more than one letter.

EFFECT OF PROPAGANDA AND INTENSIVE COUNSELLING

Table 3 Gonorrhoea in men (St Giles' Hospital)

Rate of defaults	July 1975	July 1976
After one visit	16 —=30.2% 53	8 —=17.8% 45
After two visits	19 —=35.9% 53	9 —=20% 45

$$\chi^2=7.8; P<0.025$$

The intensive patient counselling carried out in the clinic in July 1976 did appear to have a statistically significant effect in reducing both the one- and two-attendance default rates, see Table 3. However, this impression is unduly flattering to the results achieved by propaganda and counselling: for although it is true that the default rate for July 1976 was lower than that for the other three months (38%

compared with 66%, 48%, and 52%), the decrease is not significant when compared with August 1975 and August 1976. One suspects that defaulting in July 1975 was, for some reason, abnormally high, and that this made the results for July 1976 seem better than they in fact were.

The same restrictions must also apply when interpreting Tables 4, 5, and 6.

Table 4 *Gonorrhoea in men (St Giles' Hospital)*

Rate of defaults	August 1975	August 1976
After one visit	10 —= 17.9% 56	13 —= 19.7% 66
After two visits	17 —= 30.4% 56	21 —= 31.8% 66

Table 5 *Non-gonococcal urethritis in men (St Giles' Hospital)*

Rate of defaults	July 1975	July 1976
After one visit	18 —= 24.0% 75	16 —= 16.8% 95
After two visits	25 —= 33.3% 75	23 —= 24.2% 95

$$\chi^2 = 4.5; 0.20 > P > 0.10$$

Table 6 *Gonorrhoea and non-gonococcal urethritis in men (St Giles' Hospital)*

Rate of defaults	July 1975	July 1976
After one visit	34 —= 26.6% 128	24 —= 17.1% 140
After two visits	44 —= 34.4% 128	32 —= 22.9% 140

$$\chi^2 = 11.7; P < 0.01$$

Normal patient counselling was resumed in August 1976 and the results show that the default rates were similar to those of the previous year.

The reduction in one- and two-attendance default rates for men suffering from NGU was also in response to the propaganda and counselling efforts of July 1976, although not to as marked a degree as had been the case with gonorrhoea.

A possible explanation of the difference could be that gonorrhoea is regarded as more serious than NGU, not only by the patient, but also by the doctor who may therefore not emphasise the importance of continued surveillance with the same conviction as he would when dealing with gonorrhoea.

It can be seen that the propaganda and counselling programme of July 1976 reduced the one- and two-attendance default rates for the sum of patients with gonorrhoea plus patients with NGU convincingly and apparently significantly: but it must be borne in mind (*vide supra*) that the default rate for July 1975 was abnormally high and so tended to exaggerate the apparent benefit of combined counselling and propaganda in July 1976.

EFFECT OF TREATMENT SCHEDULES ON DEFAULTING

NGU trial: Kent and Canterbury Hospital and St Giles' Hospital, June to December 1974

In this therapeutic trial using two different treatments for NGU as outlined above, an interesting and unexpected finding was that the default rate in the group having weekly injections was 16.7% of 108 patients and significantly lower than in the group given oral treatment alone (33% out of 109).

$$\chi^2 = 7.1; P < 0.01$$

Discussion and conclusions

The results show that the most likely time for men to default is after the first visit, whereas women tend to 'drop out' after the second clinic attendance (Table 2). The likely explanation is that most men come to a clinic because of symptoms, whereas most women are asymptomatic at the first visit (Mahony, 1972). Men therefore tend to cease attending when symptoms clear up which is very often after taking the treatment given to them at the first visit. Women, however, have to rely for reassurance on receiving the results of bacteriological cultures which are not available until they attend the clinic for a second time. Table 2 also shows that more than half of men and women who default do so after only two attendances at the clinic. Since the response to default letters is poor (less than 15% positive response in both sexes), it follows that propaganda must be given *at the clinic* at the first and second visit. Tables 3, 5, and 6 show that such propaganda can be effective, but it has to be sustained or the defaulter rate soon reverts to the previous level (Table 4).

Moreover the results were not as good, for reasons already given, as Tables 3, 4, 5, and 6 seem to suggest. It must be concluded that counselling and propaganda, like all types of health education, do not achieve quick results, and need to be maintained to be effective.

Finally, it was found that by instructing patients to return to the clinic, not just for review and

assessment but for the specific purpose of having an injection, the default rate was significantly reduced. Since the therapeutic efficiency of both regimens (with or without an injection) was similar, it was surmised that patients were motivated to attend when treatment was valued more highly, and that injections appeared to be valued more than tablets. This point requires further elucidation and a trial with randomly allocated placebo injections is currently under way.

Our sincere thanks are due to Professor Michael Warren, Director of the Health Services Research Unit at the University of Kent at Canterbury, for his advice in designing the questionnaire employed at the Kent and Canterbury Hospital. (Default behaviour was only one facet of a comprehensive study for which the questionnaire was designed. Professor Warren's department processed the results obtained for computer analysis.)

Our thanks are also due to Mrs Sheila Baugh, secretary, and Mrs Yvonne Reeves, health worker to the Special Clinic of the Kent and Canterbury Hospital, for their help in compiling the results obtained from the questionnaire.

We would also like to thank Sister Elizabeth Schofield of St Giles' Hospital for searching the records of the Special Clinic at that hospital, and Dr Richard Hallam of the Faculty of Human Sciences in the Department of Psychology at the North East London Polytechnic who helped with statistical analysis of the results.

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Evaluation of the early morning smear investigation

P. D. SIMMONS

From the Department of Genital Medicine, St Bartholomew's Hospital, London

SUMMARY A retrospective study on early morning smears confirmed the value of this investigation for diagnosing urethritis in men. In 200 patients 108 new infections were diagnosed.

Introduction

It has long been the practice in clinics for sexually transmitted diseases (STDs) to ask men attending with a complaint of urethral discharge and/or dysuria to hold their urine for several hours before examination. This allows secretions to accumulate for clinical and microbiological examination. It is believed that the longer the interval between micturition and examination, the more accurate are the results. Men may also be asked to return early in the morning holding their urine overnight for further investigation of suspected urethritis (King and Nicol, 1975). Rodin (1971) showed that such early morning examination of apparently healthy men may reveal otherwise undetectable urethritis.

Method

Two hundred men attending St Bartholomew's Hospital between September 1976 and March 1977, with genitourinary symptoms for whom no firm diagnosis had been made on the first visit, were asked to come for an early morning smear (EMS) and culture. They attended between 8.30 and 9.0 a.m., having micturated just before retiring to bed the previous night. They were advised to restrict their fluid intake from 8.0 p.m. that night. All men were examined in accordance with the method described by Alani *et al.* (1977) with the exception that no *Chlamydia* cultures were taken during the present series. If a change in diagnosis from non-specific urethritis (NSU) to gonorrhoea resulted, or if a patient with no leucocytes on the original Gram-stained smear had more than 10 leucocytes per high power field on 20 fields in the EMS, the outcome was rated as successful. It is not our policy to treat a patient in whom the only abnormality is the

presence of threads in the first urine sample passed, as no culture for gonococci is then available.

Results

The reasons for attendance and other factors were assessed retrospectively in the 200 patients (Table 1). One hundred and eight new infections were diagnosed: five were gonorrhoea and 103 NSU (Table 1). Seventy-eight patients had no sign of urethritis and in 14 patients no further information was obtained; the reasons for requesting these investigations were not obvious.

On the first visit urine had been held for a mean of 2.5 hours, and on the EMS visit for a mean of 9.7 hours. The mean time between the first visit and EMS was 4.6 days. One hundred and thirteen (56%) patients attended for EMS within three days of their first visit and contributed 67 extra diagnoses, 73 patients came between four and 10 days after their first visit, and 14 patients were unable to attend for more than 10 days. The success rate fell from 67% when attendance was within three days to 47% when it was more than three days. Only three (8.6%) of 35 patients complained of urinary symptoms and had an abnormal first glass of urine without an infection being found on EMS (Table 2).

Discussion

Early morning investigation ensures a period of continence of at least six hours, assuming that the patient does not suffer from nocturia. Many find it easier to hold urine overnight than for a comparable period during the day. Approximately 10% of patients defaulted from their EMS appointments either because of their inability to retain urine for the test or for other reasons. Rodin (1971) found in his series of 88 asymptomatic men investigated by EMS that 11 (12.5%) had NSU. All the infected patients had abnormal first glass urine samples; he did not give details of the urine appearance on first

Table 1 Results of investigation on 200 men

Reason for request	No. of patients	Diagnosis					
		Gonorrhoea		NSU		No infection	
		No.	%	No.	%	No.	%
Complaint of urethral discharge	56	—	—	37	(66.1)	19	(33.9)
Dysuria	32	1	(3.1)	19	(59.4)	12	(37.5)
Requested check-up	15	—	—	5	(33.3)	10	(66.7)
Reason not obvious	15	—	—	1	(6.7)	*	—
Possible gonorrhoea†	12	4	(33.3)	6	(50)	2	(16.7)
Urinary threads/specks	20	—	—	17	(85)	3	(15)
Penile irritation/pain	19	—	—	7	(37.8)	12	(62.2)
Vague symptoms	9	—	—	3	(33.3)	6	(66.7)
Frequency	6	—	—	—	—	6	(100)
Loin pain (sterile MSU)	1	—	—	—	—	1	(100)
Previous 'cystitis'	1	—	—	1	(100)	—	—
Possible Reiter's syndrome	5	—	—	2	(40)	3	(60)
Trichomoniasis contact	5	—	—	3	(60)	2	(40)
NSU contact	4	—	—	2	(50)	2	(50)
	200	5		103		78	

*No further information from 14

†A diagnosis of gonorrhoea contemplated because of contact's diagnosis, suspicious Gram-stained slide, or time since infection

All percentages relate to number of requests for that reason

Table 2 Comparison of results between the first and second visit

Patients	Findings in first urine at first visit		Urethritis at EMS	
			No.	%
All	Clear	136	67	(49.3)
	Abnormal	64	55	(85.9)
			$\chi^2 = 23.1$	$P < 0.001$
Without symptoms	Clear	34	18	(52.9)
	Abnormal	29	23	(79.3)
			$\chi^2 = 3.1$	$P = 0.054$
With symptoms	Clear	102	49	(48.0)
	Abnormal	35	32	(91.4)
			$\chi^2 = 18.5$	$P < 0.001$

attendance. However, Earle (1977) showed that in a small sample of women, the number of leucocytes found per microscopic field was not increased when the patient held her urine for over two hours. Furthermore it has been shown that the isolation of *Chlamydia* from the male urethra seems not to be related to time since micturition (Oriel *et al.*, 1972), but until routine *Chlamydia* culture is undertaken at all clinics and a rapid isolation service provided, physicians will continue to rely on the presence of excess leucocytes to make the diagnosis of NSU. Judson *et al.* (1977) have shown that recent micturition does not affect the detection of urethral gonorrhoea.

The results at this clinic show that patients attending with symptoms and abnormalities in the first glass of urine should be carefully screened for infection with the aid of an EMS investigation. New cases of urethritis will also be found if asymptomatic

patients with abnormal urine are screened in this way. The number of patients attending most clinics probably precludes an EMS investigation on all men attending the clinic requesting an examination for STDs. To increase the chances of finding urine abnormalities, we ask all patients at no particular risk for a 'first-catch' early morning urine sample. For those in whom leucocytes are found on centrifugation and microscopical examination of this urine, an EMS investigation is arranged.

I should like to acknowledge the help of Miss Monica Leighton of the Medical Computing and Statistics Unit, St Bartholomew's Hospital.

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Asymptomatic gonococcal urethritis in a male transsexual female

NICHOLAS J. FIUMARA AND SHAHLA ASVADI

*From the Division of Communicable and Venereal Diseases,
Massachusetts Department of Public Health*

In a previous paper (Fiumara and Di Mattia, 1973) a male transsexual patient was described with symptomatic gonococcal urethritis. Since then, after an interval of four years, a second patient has been found, who is asymptotically infected. The incidence of surgical sex reassignment of the male transsexual in the United States is not available, but the operation is being performed at many university hospitals and it is expected that physicians will occasionally see such patients. In an attempt to understand more of the pathogenesis of gonorrhoea in these patients, the following case reports may be of interest.

Case reports

A 37-year-old, White, divorced man came to the Skin Clinic of the Boston Dispensary on 14 July 1977 with dysuria and a purulent urethral discharge of five days' duration. The Gram stain revealed many Gram-negative intracellular and extracellular diplococci and a Thayer-Martin culture was positive for gonococci recognised by morphology and oxidase reaction.

The patient was treated with 4.8 megaunits of aqueous procaine penicillin G. The blood rapid plasma reagin circle card test (RPRCT) was non-reactive. He named two female sexual contacts—Alice, a 26-year-old, White, divorced woman (contact on 4 July) and Jane, a 32-year-old, single woman (contact on 9 July).

Alice came in for examination on 18 July. She was found to be infected with gonorrhoea, her culture was positive, and she was treated.

Jane presented on 21 July. She was a 32-year-old male transsexed female who had had genital reconstructive surgery two years earlier. On examination she was a tall big-boned woman, well proportioned, with soft, white, non-hairy skin, well developed breasts (the result of silicone injections and oestrogen), male pattern pubic hair, and reconstructed

external sexual organs of female appearance (Fig. 1). Her hair was long and well groomed and her voice was pleasantly deep. There was no urethral discharge and even after milking the urethra through the new vagina no discharge could be expressed. Nevertheless a smear and culture were taken (Fig. 2). The Gram stain revealed few polymorphonuclear leucocytes and few Gram-negative intracellular diplococci. The culture on Thayer-Martin plate was positive for gonococci. Jane was treated with 4.8 megaunits of aqueous procaine penicillin. One week later the urethral smear and culture were negative.



Fig. 1 *The reconstructed external sexual organs of female appearance*



Fig. 2 *Milking the urethra through the new vagina*

Address for reprints: Dr N. J. Fiumara, Director, Division of Communicable Diseases, Massachusetts Department of Public Health, 600 Washington Street, Boston 02111, Massachusetts, USA

Received for publication 21 September 1977

Discussion

The previous report (Fiumara and Di Mattia, 1973) summarised the psychological nature of the male transsexed patient and the surgical procedures; the reconstructed vagina is lined with stratified squamous epithelium which is resistant to infection with gonorrhoea (Fiumara, 1972). There is constant desquamation of the keratin layer and patients are advised to clean the vagina with a little oil on a swab to prevent irritation and infection. Gonococcal infection should be looked for in the urethra and specimens for laboratory examination must be taken from this site.

Unlike our other two patients (Fiumara and Di Mattia, 1973; Fiumara, unpublished), Jane had a small vaginal introitus (Fig. 3). In discussing this with her she remarked it did not interfere with sexual intercourse because she lubricated it beforehand and it stretched without discomfort.

It is not surprising that asymptomatic gonorrhoea was found in a male transsexed female. Between 3 and 15% of infected males may be asymptomatic, Handsfield *et al.* (1974). The male transsexed female is anatomically a male with the spongy portion of the penis amputated at the proximal end. Therefore, this portion of the urethra is lined with columnar epithelium which readily supports the invasion of the gonococcus. What the attack rate may be in these male transsexed females on exposure



Fig. 3 Vaginal introitus in one patient

to a male patient with gonorrhoea is unknown. As physicians gain more experience in examining, diagnosing, and treating male transsexed females, the epidemiology of gonorrhoea in such patients will become apparent.

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Abstracts

These selected abstracts and titles from the world literature are arranged in the following sections:

Syphilis and other treponematoses

(Clinical and therapy; serology and biological false positive phenomenon; pathology and experimental)

Gonorrhoea

(Clinical; microbiology; therapy)

Non-specific genital infection

Reiter's disease

Trichomoniasis

Candidosis

Genital herpes

Other sexually transmitted diseases

Public health and social aspects

Miscellaneous

Syphilis and other treponematoses (Clinical and therapy)

The incidence of treponemal disease in adult men at Loitokitok, Kajiado District

J. D. GATENBY DAVIS AND C. HINREINER (1977). *East African Medical Journal*, 54, 273-275

Sera were collected from 148 adult males, aged between 18 and 48 years, who attended the Loitokitok Hospital in the Kajiado District of southern Kenya. VDRL and TPHA tests were performed; 11 sera were reactive in both tests and 12 sera were reactive in the TPHA test only. FTA-ABS tests were positive on all, so that serological evidence of treponemal infection was present in 15.4% of the patients surveyed. Reactivity was equally divided between patients of the pastoral group of Masai and the non-pastoral Bantu. The patients were not examined, nor were histories taken, so that it was not known whether the results reflected venereal syphilis or the endemic non-venereal form. The simplicity and specificity of the TPHA test make it of especial value in epidemiological surveys of this kind.

A. E. Wilkinson

[Reprinted from *Abstracts on Hygiene*, by permission of the Editor.]

Return to normal of Argyll-Robertson pupils after treatment

F. M. LANIGAN-O'KEEFE (1977).

British Medical Journal, 2, 1191-1192

Syphilis (Serology and biological false positive phenomenon)

Rapid detection of specific antitreponemal antibodies by counterimmunoelectrophoresis using an extract of *Treponema pallidum*

R. GHINSBERG, B. W. GRUNBAUM, AND G. BLUMSTEIN (1977).

Israel Journal of Medical Sciences, 13, 557-560

The antigen was prepared by ultrasonic disruption of a suspension containing 10^7 *Treponema pallidum* per ml in 0.02 mol/l phosphate buffer, pH 7.0. Wells 3 mm in diameter with 5 mm between centres were cut in 0.85% agarose gel in 0.06 mol/l barbital buffer, pH 8.2. Serum was placed in the anodal and antigen in the cathodal wells and a constant voltage of 60 V at 4 mA passed for 45 minutes.

Comparison of the *T. pallidum* counterimmunoelectrophoresis (TPCIE) and FTA-ABS tests on 400 sera from patients with suspected syphilis showed agreement in 96%. Of the 16 sera which gave discrepant results the TPCIE test was positive in four and the FTA-ABS test in 12; syphilis was diagnosed on clinical grounds in 15 of these patients. In a comparison of the TPCIE and Reiter protein complement-fixation tests on 400 additional sera the results agreed in 86%. In tests on sera from 17 patients with untreated early (nine) or latent (eight) syphilis the FTA-ABS test was positive in all, the TPCIE in 14, and the RPCFT in 10. Thirty-nine sera which were thought to have given false positive RPCFT results

were all negative with the FTA-ABS and TPCIE tests. The TPCIE test is thought to offer the advantages of speed, simplicity, and economy over the FTA-ABS test but to be slightly less sensitive in the detection of primary syphilis.

A. E. Wilkinson

Syphilis (Pathology and experimental)

Parasitism by virulent *Treponema pallidum* of host cell surfaces

N. S. HAYES, K. E. MUSE, A. M. COLLIER, AND J. B. BASEMA (1977).

Infection and Immunity, 17, 184-186

The attachment of *Treponema pallidum* was four times higher for monolayers of rabbit testicular cells than for one specified line of human epithelial cells. There was greater cellular adherence when incubated at 37°C than at 33°C, 25°C, or 4°C and the effect was dependent on the quantity of added treponemes. At the most, two hours were necessary for maximal attachment. Once attached, the treponemes remained motile, but were anchored by a 'terminal tapered structure'. Exposure of cell cultures to 7000-8000 rad or to the mitogens, phytohaemagglutinin, concanavalin A, or pokeweed failed to inhibit treponemal adhesion. The cell cultures were also treated with detergents, certain enzymes, and metabolic inhibitors—again without prevention of adhesion. No treponemal adherence was found to glass particles of 5-125 µm or diethylaminoethyl-Sephadex A-50 swollen beads 40-120 µm. One per cent rabbit serum or calf

serum-coated Sephadex beads likewise failed to enhance adherence.

Heat or formalin-treated treponemes failed to adhere to cell monolayers. If incubated for 21 hours at 33°C, they showed a 75% reduction in adhesion. Similarly, high doses of potassium cyanide or sodium deoxycholate and, to a lesser extent, digitonin inhibited adhesion and motility. Exclusion of calcium and magnesium ions by ethylenediaminetetraacetate implied that adhesion was not dependent on divalent cations. No cellular adhesion was shown with pathogenic 'Reiter' treponemes. Monolayer cultures with treponemes adhering to the cell surfaces were refractory to further treponemal adhesion. Fifty per cent inhibition of adhesion was produced by preincubation with rabbit sera taken 135 days after infection with *Treponema pallidum*. These sera had a fluorescent treponemal antibody—ABS titre of 3125.

[The authors imply there is a specific cellular attachment for *Treponema pallidum*. However, this suggestion would have been more convincing if the possibility of host antibody, perhaps on the surface of the spirochaete, cross-reacting with the cells in the monolayer, particularly with the rabbit testicular cells, had been excluded.]

D. J. M. Wright

Development of macrophage migration inhibition in rabbits infected with virulent *Treponema pallidum*

C. S. PAVIA, J. D. FOLDS, AND J. B. BASEMAN (1977). *Infection and Immunity*, 17, 651–654

Terminal electron transport in *Treponema pallidum*

P. G. LYSKO AND C. D. COX (1977). *Infection and Immunity*, 16, 885

Gonorrhoea (Clinical)

Gonorrhoea screening in male consorts of women with pelvic infection

L. C. GILSTRAP, W. P. N. HERBERT, G. CUNNINGHAM, J. C. HAUTH, AND H. G. VAN PATTEN (1977). *Journal of the American Medical Association*, 238, 965–966

This report describes a study of men as a reservoir of gonorrhoea and of their role in pelvic infections in women. Urethral cultures were taken from 161 male contacts of 100 women with acute gonococcal pelvic inflammatory disease (PID). Positive cultures for *N. gonorrhoeae* were obtained from 63 contacts (39%), of whom 14 (22%) were asymptomatic. It was also noted that 52% of these women had at least one sexual contact from whom *N. gonorrhoeae* was isolated. A group of 69 male contacts of 46 women with non-gonococcal PID was examined. Cultures of *N. gonorrhoeae* were yielded by 10 (15%), three of whom were asymptomatic.

In view of these findings the authors rightly recommend that all male sexual contacts of women with acute PID should be examined, with cultures for *N. gonorrhoeae*.

C. S. Ratnatunga

Futility of routine culture of hysterectomy specimens for gonorrhea

S. BURROWS (1977). *American Journal of Obstetrics and Gynecology*, 129, 339

Resistant gonococcal infection from an intrauterine contraceptive device

E. E. DASHOW AND A. S. LLORENS (1977). *American Journal of Obstetrics and Gynecology*, 129, 230

Gonorrhoea (Microbiology)

Ultrastructural study of cervical gonorrhea

B. A. EVANS (1977). *Journal of Infectious Diseases*, 136, 248–254

An ultrastructural examination of the squamocolumnar junction of the cervix of patients infected with *Neisseria gonorrhoeae* is described. Gonococci were found to become firmly attached to stratified squamous epithelium, a process that appeared to be initiated by activity of the cytoplasmic membrane of superficial squames. By contrast, gonococci were not found attached to, or even closely associated with, mucus-secreting columnar epithelium. Gonococcal growth, as evidenced by numbers of organisms and surface vesicle formation, appeared most

active in cervical secretions, chiefly on exfoliated squames, but also lying free. Survival after phagocytosis by polymorphonuclear leucocytes was found not to be unique to gonococci, and it is suggested that continual phagocytic recycling minimises the significance of this occurrence. It seems probable that persistence of gonorrhoea in the female depends upon the adherence of gonococci to stratified epithelium, where they are protected from phagocytosis, and the infectivity of gonorrhoea arises from the ability of gonococci to divide rapidly on the surface of exfoliated squames, from where they are released into secretions.

Author's summary

Studies on the mechanism of pathogenicity of *Neisseria gonorrhoeae*

P. NOVOTNY, J. A. SHORT, M. HUGHES, J. J. MILLER, C. SYRETT, W. H. TURNER, J. R. W. HARRIS, AND I. P. B. MACLENNAN (1977). *Journal of Medical Microbiology*, 10, 347–365

Gonococci in pus appear in specific clusters in which they are surrounded by organelles and granules derived from the host cells in which they multiplied. These clusters have been named *infectious units* because (1) the cocci multiply within them, (2) the whole complex makes contact with epithelial cells, (3) the cocci in the units are not recognised by polymorphs as long as the coating of granules is dense enough, and (4) the cocci are probably protected against humoral defence mechanisms. During multiplication of bacteria in infectious units, soluble antigenic material is probably produced. No morphological evidence of multiplication of gonococci outside infectious units was observed in pus from patients with gonorrhoea. Attempts to reproduce typical infection units in animal models have so far failed.

The identity of the surrounding granular coating was established morphologically and with appropriate sera labelled with ¹²⁵I and horseradish peroxidase. It is proposed that the mechanism of gonococcal pathogenicity is primarily based on the internal disorganisation of the regulatory mechanism of human macrophages. A sequence of events in the infection is discussed.

The anatomical changes observed in subcutaneously implanted plastic chambers in guinea-pigs infected with gonococci may be attributable to partial interference

with the internal regulation of phagocytes. The effect of drugs on bacterial counts in the chamber cavity was studied. Under the influence of colchicine the number of colony-forming units increased 100 times within 8 hours; vinblastine was without effect. Neither of these drugs had any effect on the growth of gonococci *in vitro*. In studies by immune electron microscopy with sera from patients with gonococcal septicaemia, IgG was shown to react strongly with the gonococcal surface, free endotoxin, ring structures (pits) liberated from the lipopolysaccharide backbone and with unspecified soluble antigenic material. The detection of plated antibodies in these sera was rare.

Authors' summary

Surface components affecting interactions between *Neisseria gonorrhoeae* and eucaryotic cells
J. SWANSON (1977).

Journal of Infectious Diseases, 136, S138-S143

Interactions of *Neisseria gonorrhoeae* with non-leucocytic eucaryotic cells including tissue culture cells (either primary or continuous lines), human sperm, and buccal mucosal cells appear to be influenced primarily by the presence or absence of pili on the bacteria. In this context, piliation enhances attachment to or association of micro-organisms with the eucaryotic cells. Pili, on the other hand, appear to reduce interaction of *N. gonorrhoeae* with mouse peritoneal macrophages. Other groups have demonstrated that pili reduce phagocytosis of gonococci by neutrophils, but in our studies this effect is secondary to that dependent on 'leucocyte-association' factor. The presence of such a factor is correlated with a particular protein found by polyacrylamide electrophoresis. Adherence among *N. gonorrhoeae* varies and results in colony forms of different colouration or aggregation characteristics. These different colouration forms correlate with the protein patterns of gonococci as found in polyacrylamide electrophoresis and also with the susceptibility of gonococci to killing by trypsin.

Author's summary

Rapid method for auxotyping multiple strains of *Neisseria gonorrhoeae*

H. B. SHORT, V. B. PLOSCOWE, J. A. WEISS, AND F. E. YOUNG (1977).

Journal of Clinical Microbiology, 6, 244

A rapid method for auxotyping strains was developed that uses microtitre plates. This miniplate technique enables rapid identification of major auxotypes present in clinical strains. Additional growth requirements can be identified by adding individual amino-acid supplements to complete gonococcal genetic medium. Analysis of eight clones from 40 patients revealed that 10 had more than one auxotype. Deoxyribonucleic acid-mediated transformation can be used to establish whether the strains with apparently more than one auxotype are defective in the same locus in each of the involved biosynthetic pathways. Selection of more than one clone is required in precise epidemiological studies.

Authors' summary

Auxotypes and penicillin susceptibilities of *Neisseria gonorrhoeae* isolated from patients with gonorrhoea involving two or more sites

B. W. CATLIN AND P. J. PACE (1977).

Antimicrobial Agents and Chemotherapy, 12, 147-156

The auxotype and MIC of penicillin were determined for 181 isolates from different sites in 84 patients with uncomplicated gonorrhoea. Auxotypes 1, 2, and 3 comprised 27%, 36%, and 24% of the isolates, but it was also possible to distinguish strains within an auxotype by differences in their susceptibility to antibiotics. Pairs of isolates from three patients showed different auxotypes and two of these pairs showed differences in their resistance to a range of antibiotics. This suggests that these three patients were each infected by two distinct strains of gonococci. In three other patients the paired strains showed slight differences in the MIC of penicillin, but their auxotypes and degree of resistance to other antibiotics were identical. Gonococci from eight couples with uncomplicated gonorrhoea showed the same auxotype and susceptibility to penicillin.

Twenty-one isolates from multiple sites in nine patients with disseminated gonococcal infection and three of their contacts were tested. All except one strain required arginine, hypoxanthine, and uracil for growth and were very sensitive to penicillin. Strains from different sites in the individual patients and from one contact had the same auxotype and resistance to penicillin. One patient had two contacts, the isolates were alike

in their antibiotic resistance profiles and requirement for arginine, hypoxanthine, and uracil, but the strain from one contact also required thiamine pyrophosphate for growth.

The biosynthetically less competent strains requiring arginine, hypoxanthine, and uracil seem to be less well adapted to growth and survival in the oropharynx than the nutritionally less demanding strains of auxotypes 1, 2, and 3.

A. E. Wilkinson

Conjugal transfer of R. plasmids in *Neisseria gonorrhoeae*

M. ROBERTS AND S. FALKOW (1977).

Nature, 266, 630-631

The molecular weights were determined of plasmids derived from lysates of 13 strains of *N. gonorrhoeae* which showed β -lactamase activity, that is, penicillin resistance. The molecular weights were measured by comparing differences in mobility of the plasmids in agarose gel after electrophoresis and also by measuring the contour length of open circular DNA in the electron microscope. All strains contained plasmids of 2.5 megadaltons, nine Far Eastern strains had plasmids of 4.4 megadaltons in addition, while the four strains isolated in Great Britain carried plasmids of 3.2 megadaltons. In three of the Far Eastern strains a further plasmid of 24.5 megadaltons was found.

Matings were accomplished by a variation of the filter method (not published). The donor strains were: one Far Eastern strain with high and low molecular weight plasmids and two strains containing only the lower molecular weight plasmids, one from the Far East and one from Great Britain. A penicillin sensitive recipient strain of *N. gonorrhoeae* was used which, unlike the donor strains, was resistant to nalidixic acid and rifampicin but required proline. Transfer of penicillin resistance without changes in the sensitivity to other antibiotics and alteration in proline requirement occurred only in the strain containing the large 24.5 megadalton cryptic plasmid. The strain also transferred β -lactamase activity to an ampicillin-sensitive *Escherichia coli*, the β -lactamase activity being determined by the chromogenic cephalosporin substrate. The conjugal transfer of β -lactamase activity is not accompanied by the finding of the 24.5 megadalton plasmid in the

recipient strain, although the low molecular weight plasmid is present. The plasmid transfer was not affected by pancreatic DNase but no data were provided for this experiment. All the *Neisseria* whether donor or recipient exhibited non-piliated T_4 colonial morphology (T_4 colonies are not normally competent for DNA transformation). A T_1 *N. gonorrhoeae* strain showed transformation using proline dependence as a marker with bulk DNA but not plasmid DNA using Catlin defined media. Kirven and Thornsberry (1977) have also shown conjugal transfer of DNA to other strains of *Neisseria* and to *E. coli*. They also provided data for the failure of DNase to influence conjugal transfer of genetic material.

Similar independent observations have been made by Eisenstein *et al.* (1977) who demonstrated conjugal transfer of β -lactamase activity using two penicillin resistant strains of *Neisseria* to two other strains of *Neisseria*, two *E. coli*, and also to *Neisseria flava*. They also provided experimental evidence to show that there was no phage transduction by showing that sterile filtrates of *Neisseria* broth cultures derived from penicillin resistant strains failed to transfer resistance.

These experiments indicate that conjugation but not transformation or transduction is the likely means of transfer of β -lactamase activity, also that the heavy cryptic plasmid exhibits sex factor activity. The inability to find the heavy plasmid in the British or recipient strains suggests that covalent linkage with the heavy plasmid may not be necessary. The subject is reviewed by J. R. Saunders (1977) in *Nature*, 266, 586 and the paper should be read in conjunction with that by L. A. Kirven and C. Thornsberry (1977), *Antimicrobial Agents and Chemotherapy*, 11, 1004, and by B. I. Eisenstein, T. Sox, E. Biswas, E. Blackman, and P. F. Sparling (1977), *Science*, 195, 998.

D. J. M. Wright

Molecular characterisation of two beta-lactamase-specifying plasmids isolated from *Neisseria gonorrhoeae*

M. ROBERTS, L. P. ELWELL, AND S. FALCOW (1977). *Journal of Bacteriology*, 131, 557-563

Two non-conjugative gonococcal R-plasmids of molecular mass of 4.4 megadaltons and 3.2 megadaltons were studied. Both these plasmids included β -lactamase activity. The 4.4 megadalton plasmid was originally derived from a Far Eastern

strain of *N. gonorrhoeae* while the 3.2 megadalton plasmid originated from the United Kingdom. The guanine-plus-cytosine content of these plasmids approximated to the 0.4 mol fraction. It was suggested that this value was significantly different from the guanine-plus-cytosine content of the gonococcal chromosomal deoxyribonucleic acid or of the 'indigenous' 24.5 megadalton or 2.4 megadalton plasmids. This in turn indicated that both the β -lactamase associated plasmids were not a product of a transpositional event. However, their similarity to the 4.1 megadalton plasmid derived from the β -lactamase producing strain of *Haemophilus parainfluenzae* was reinforced by the finding of a common core of deoxyribonucleic acid—deoxyribonucleic acid polynucleotide sequence of 64 to 70% of whole cell extracts of the appropriate *N. gonorrhoeae* strain, *E. coli* transformants containing the neisserial β -lactamase associated R-plasmids and an *H. influenzae* transformant containing the *H. parainfluenzae* β -lactamase R-plasmid. It was further suggested that the gonococcal R-plasmids are not necessarily derived from the *Haemophilus* species, but since they may be transformed into *E. coli* and contain 34 to 40% of the transposable *E. coli* deoxyribonucleic acid sequence TN2, the structural gene of the ubiquitous TEM β -lactamases, the β -lactamase plasmids, are derived from the enteric pool. The implication is that there has been an R-plasmid intrusion across a previously invulnerable biological barrier protecting *Neisseria* and *Haemophilus*. The appearance of β -lactamase gonococcal R-plasmids with different molecular masses presumably arose from a 1.2 megadalton deletion of a 4.4 megadalton R-plasmid.

In vitro removal of selective antibiotic pressure on gonococcal R-plasmid *E. coli* transformants showed a 45% loss of 4.4 megadalton plasmid at 60 hours, which was stable thereafter, but a 99.9% loss at 120 hours of the 3.2 megadalton plasmid. This parallels the slight increase in plasmid loss in clinical isolates of penicillin-resistant strains of *N. gonorrhoeae* from the United Kingdom (36 to 99.9% loss) whereas the Far Eastern strains showed a 30 to 98% loss. This in part may reflect that the United Kingdom isolates were collected early in the epidemic. It is interesting to speculate what effect the conjugative 24.5 megadalton plasmid has on the possible host parasite relationship in man, as this plasmid occurs in 50% of

the penicillin-resistant strains of *Neisseria gonorrhoeae* as compared with only 5% of sensitive strains.

D. J. M. Wright

Rapid penicillinase paper strip test for detection of beta-lactamase-producing *Haemophilus influenzae* and *Neisseria gonorrhoeae*

J. H. JORGENSEN, J. C. LEE, AND G. A. ALEXANDER (1977).

Antimicrobial Agents and Chemotherapy, 11, 1087-1088

Strips of Whatman filter paper no. 3 about 5 by 1 cm in size are immersed in a solution of 0.2% soluble starch (BBL) and 1% potassium penicillin G, dried at room temperature for 2 hours and stored at -20°C ; they remain stable for at least a year.

For testing, a strip is placed in a sterile Petri dish, moistened with Gram's or Lugol's iodine and the excess poured off. Growth from about 10 colonies is smeared on the strip in an area about 5 mm in diameter. If β -lactamase is produced, the dark purple colour of the strip becomes white round the area inoculated within a minute; this is due to the production of penicilloic acid which converts iodine to iodide. In a negative test the strip remains purple or becomes slightly yellow at the site of inoculation.

The test gave satisfactory results with 100 isolates of *Haemophilus influenzae* and 27 of *Neisseria gonorrhoeae*.

A. E. Wilkinson

Evidence for two distinct types of penicillinase-producing *Neisseria gonorrhoeae*

P. L. PERINE, W. SCHALLA, M. S. SIEGEL, C. THORNSBERRY, J. BIDDLE, K-H WONG, AND S. E. THOMPSON (1977). *Lancet*, 2, 993-995

Since their recognition early in 1976, penicillinase—(β -lactamase) producing *Neisseria gonorrhoeae* (PPNG) have been isolated in more than 15 countries. Most strains isolated in or epidemiologically linked with the Far East are relatively resistant to tetracycline *in vitro*, are phenotypically wild-type or proline-dependent auxotypes, and carry a plasmid with a molecular weight of 5.8 megadaltons coding for β -lactamase production. In contrast, PPNG epidemiologically

linked with West Africa are more susceptible to tetracycline, require arginine for growth, and their gene coding for β -lactamase synthesis is contained in a smaller 3.2 megadalton plasmid. Moreover, 43% of the Far Eastern strains, but none of those from West Africa, have an additional 24.5 megadalton conjugative plasmid which transfers the β -lactamase R factor(s) to other gonococci. The presence of this conjugative plasmid may explain the relatively high prevalence of PPNG in certain areas of the Far East.

Authors' summary

Penicillin sensitivity and serum resistance are independent attributes of strains of *Neisseria gonorrhoeae* causing disseminated gonococcal infection

B. I. EISENSTEIN, T. J. LEE, and P. F. SPARLING (1977).

Infection and Immunity, 15, 834-870

This is the most comprehensive study yet to examine the inter-relationship between various phenotypic characteristics of *Neisseria gonorrhoeae* and its virulence as manifest by disseminated infection (DGI). The authors set out with the plausible hypothesis that mutation to antibiotic resistance, or adaptation from the 'wild type', might be expected to correlate with decreasing virulence. High correlations are demonstrated independently both with penicillin sensitivity (Pen^S) and resistance to the bactericidal effect of pooled human serum (serum^R).^{*} Recent reports attributing a high percentage of DGI cases to strains of gonococci with particular nutritional requirements (auxotype AHU⁻) are explained by this strain usually being penicillin sensitive, rather than by any inherent mutational effect of nutritional requirement on virulence. As regards the correlation of antibiotic sensitivity and serum resistance with virulence, the authors draw on support from other workers reporting similar phenomena in enteric bacteria, and note that all these properties are likely to be related to the bacterial surface.

Finally, the increasing prevalence of AHU⁻ strains in uncomplicated gonorrhoea is suggested as being due to the slow growing nature reported by Morello *et al.* (1976), which renders them unaffected by short-acting high levels of antibiotic as are currently in vogue for treatment.

^{*}Sensitivity to streptomycin (Str^S) and toxic agar (Toxic agar^S) bore no significant

relationship to virulence, but there was a correlation with strains of intermediate sensitivity to erythromycin (Ery^{WT}), which comprise the 'wild types', those above and below often being genetic mutants.

Brian Evans

Protection against infection with *Neisseria gonorrhoeae* by immunization with outer membrane protein complex and purified pili

T. M. BUCHANAN, W. A. PEARCE, G. K. SCHOOLNIK, AND R. J. ARKO (1977). *Journal of Infectious Diseases*, 136, Supplement, 132-137

Some of the antigens that are capable of producing strain-related immunity to gonococcal infection in the guinea-pig are located on the outer membrane of *Neisseria gonorrhoeae*. This finding has been demonstrated by immunisation of guinea-pigs with isolated outer membranes from two different strains of *N. gonorrhoeae* prior to challenge. Isolated principal outer membrane protein complex proved a better protective immunogen than purified pili from the same strain of *N. gonorrhoeae*. Principal outer membrane protein appears to react in antibody-complement-mediated killing of gonococci, whereas antibodies to pili are only weakly bactericidal. Pili-mediated attachment of *N. gonorrhoeae* to human cells is inhibited by antibodies to pili, and maximal inhibition occurs when antibodies are directed at pili antigenically identical with the pili mediating the attachment.

Authors' summary

Immunity to gonococcal infection induced by vaccination with isolated outer membranes of *Neisseria gonorrhoeae* in guinea-pigs

T. M. BUCHANAN AND R. J. ARKO (1977). *Journal of Infectious Diseases* 135, 879-887

Purified outer membranes from *Neisseria gonorrhoeae* strain M or B were prepared. Gonococcal outer membranes were pure as evidenced by banding in sucrose gradients at a density of 1.23 g/cm³, simple protein composition patterns when analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, low succinic dehydrogenase levels, and few precipitin peaks obtained in two-dimensional immunoelectrophoresis against hy-

perimmune serum to gonococcal sonicates. Isolated outer membranes administered as antigen to guinea-pigs produced strain-related protection against gonococcal infection in amounts <1% of the weight of whole gonococci required to produce comparable protection in chimpanzees. One-hundred per cent (18 of 18) and 95% of control guinea-pigs (19 of 20) were infected when challenged with M and B strain gonococci respectively. In contrast, 26% (eight of 31) and 94% (16 of 17) of guinea-pigs immunised with M strain outer membrane were infected when challenged with comparable inocula of M and B strain gonococci respectively. Similarly, 25% (two of eight) and 100% (six of six) of animals immunised with B strain outer membrane were infected when challenged with comparable inocula of B and M strain gonococci respectively. These results suggest that antigens capable of inducing strain-related protection to gonococcal infection are present within the gonococcal outer membrane.

Authors' summary

Imferon agar: improved medium for isolation of pathogenic *Neisseria*

S. M. PAYNE AND R. A. FINKELSTEIN (1977). *Journal of Clinical Microbiology*, 6, 293-297

Cell envelope of *Neisseria gonorrhoeae*: relationship between autolysis in buffer and the hydrolysis of peptidoglycan

W. S. WEGENER, B. H. HEBELER, AND S. A. MORSE (1977).

Infection and Immunity, 18, 210-225

Radiometric detection of carbohydrate catabolism by pathogenic *Neisseria*

F. R. COX, M. E. COX, J. R. MARTIN, AND U. H. STÖER (1977). *Journal of Clinical Microbiology*, 6, 310-311

Inhibitory action of fatty acids on the growth of *Neisseria gonorrhoeae*

R. D. MILLER, K. E. BROWN, AND S. A. MORSE (1977).

Infection and Immunity, 17, 303-318

Reversion of Kellogg's colonial types of *Neisseria gonorrhoeae* in liquid medium

S. HAFIZ, M. G. MCENTEGART, AND A. E. JEPHCOTT (1977). *Journal of Medical Microbiology*, 10, 377-380

Virulence and immunogenicity of Types 1 and 3 *Neisseria gonorrhoeae* in guinea-pig subcutaneous chambers

R. J. ARKO, K. H. WONG, S. E. THOMPSON, W. O. SCHALLA, AND L. C. LOGAN (1977). *Canadian Journal of Microbiology*, **23**, 1261-1265

β -Lactamase producing gonococcus from Ghana

J. BARROW AND I. PHILLIPS (Letter) (1977). *Lancet*, **2**, 1023

Purification of *Neisseria gonorrhoeae* surface I-antigen

N. C. CHEN, K. KAMEI, J. ZUCKERMAN, AND H. A. GAAFER (1977). *Infection and Immunity*, **18**, 230

Immunity to gonococcal infection induced by vaccination with isolated outer membranes of *Neisseria gonorrhoeae* in guinea-pigs

T. M. BUCHANAN AND R. J. ARKO (1977). *Journal of Infectious Diseases*, **135**, 879-887

A vaccine against gonorrhoea? (Leading Article) (1977). *British Medical Journal*, **2**, 917.

Perihepatitis and hepatitis as complications of experimental endocarditis due to *Neisseria gonorrhoeae* in the rabbit

R. L. KASPAR AND D. J. DRUTZ (1977). *Journal of Infectious Diseases*, **136**, 37-42

Gonorrhoea (Therapy)

Treatment of gonorrhoea with trimethoprim-sulphamethoxazole

W. C. ELLIOTT, G. REYNOLDS, C. THORNSBERRY, D. S. KELLOGG, H. W. JAFFE, S. T. BROWN, J. ARMSTRONG, AND M. E. REIN (1977). *Journal of Infectious Diseases*, **135**, 939-943

Two single-day treatment regimens of trimethoprim-sulphamethoxazole (TMP-SMZ) were compared with the standard penicillin regimen recommended by the US Public Health Service in the treatment of 271 men with uncomplicated gonococcal urethritis.

A group of 86 men received a single dose of nine tablets of TMP-SMZ (TMP-SMZ-9), 94 received 12 tablets of TMP-SMZ in two doses of six tablets at six-hourly intervals (TMP-SMZ-12), and 91 received 4.8 megaunits of aqueous procaine penicillin G intramuscularly with 1 g probenecid given orally (APPG). Patients were asked to return after 3-6 days for re-examination and further cultures for *N. gonorrhoeae*. A positive culture was considered to indicate treatment failure, regardless of a history of re-exposure to infection. Patients not returning for examination within 13 days were excluded from statistical analysis.

Failure rates were 15/66 (23%) with 20 defaulters in the TMP-SMZ-9 group, 14/74 (19%) with 20 defaulters in the TMP-SMZ-12 group, and 2/55 (4%) with 36 defaulters in the APPG group. The smaller number of defaulters in the TMP-SMZ groups is explained by the higher failure rate and therefore the persistence of symptoms.

MIC studies showed an association between the MICs of TMP, SMZ, TMP-SMZ, and penicillin. Gonococcal isolates from patients whose treatment with penicillin had failed showed increased MICs to penicillin and tetracycline. Retreatment of these patients with TMP-SMZ could result in even higher failure rates than those observed in this study, and therefore the drug should not be used in patients showing failures to penicillin or tetracycline.

One-day regimens of TMP-SMZ are unacceptable; higher doses might give better results, but are associated with increased side effects.

N. A. Durham

Spectinomycin-resistant *Neisseria gonorrhoeae*

C. THORNSBERRY, H. JAFFEE, T. BROWN, T. EDWARDS, J. W. BIDDLE, AND S. E. THOMPSON (1977). *Journal of the American Medical Association*, **237**, 2405-2406

A 26-year-old man with a urethral discharge was treated with 3.5 g ampicillin and 1 g probenecid orally without benefit. At his next visit five days later he received 2 g spectinomycin intramuscularly and again three days later. Although he was symptom-free at his next attendance four days later, his urethral smear still showed diplococci so he was given a further 2 g spectinomycin and started on a course of

tetracycline totalling 9.5 g. Swabs from the urethra, rectum, and pharynx were negative on two visits after treatment was completed. The gonococcus isolated before treatment was started was found to grow in the presence of 2048 μ g/ml spectinomycin hydrochloride but showed no cross-resistance with aminoglycosides and its sensitivity to other antibiotics was in no way exceptional. The authors found no evidence that this strain inactivated spectinomycin and conclude that its resistance was due to chromosomal mutation.

Pamela M. Waterworth

Comparison of erythromycin base and estolate in gonococcal urethritis

S. T. BROWN, A. H. B. PEDERSEN, AND K. K. HOLMES (1977). *Journal of the American Medical Association*, **238**, 1371-1373

Erythromycin base and erythromycin estolate were compared double-blind in a randomised trial in which 152 men with urethral gonorrhoea were given 1.5 g immediately, followed by 0.5 g four times a day for four days to a total of 9 g. This treatment failed to cure 23% of the base group and 24% of the estolate group, despite the mean serum erythromycin activity being nearly twice as high in the latter group. Gastrointestinal side effects occurred in 73% and 57% of cases respectively, but no evidence of hepatic dysfunction was found although enzymes were measured.

Erythromycin appears to have no place in the treatment of gonorrhoea today.

Brian Evans

In vitro activity of p-hydroxybenzyl penicillin (penicillin X) and five other penicillins against *Neisseria gonorrhoeae*. Comparisons of strains from patients with uncomplicated infections and from women with pelvic inflammatory disease S. G. SACKEL, S. ALPERT, B. ROSNER, W. MCCORMACK, AND M. FINLAND (1977). *Antimicrobial Agents and Chemotherapy*, **12**, 31-36

Minimum inhibitory concentrations (MICs) of six penicillins against 95 strains of *Neisseria gonorrhoeae* from patients with uncomplicated anogenital infections and 22 strains from women with pelvic inflammatory disease were determined by an agar plate dilution method, using an

inocula replicator. Against all 117 strains, the order of activity observed was: BL-P1654 > penicillin X > penicillin G > ampicillin > amoxicillin = carbenicillin. MICs against strains isolated from women with gonococcal pelvic inflammatory disease were significantly higher than those against isolates from uncomplicated infections: BL-P1654, $P < 0.001$; penicillin X, $P < 0.001$; penicillin G, $P < 0.001$; ampicillin, $P < 0.001$; and amoxicillin, $P < 0.05$. MICs of penicillin G were $> 0.125 \mu\text{g/ml}$ against 33 (36%) of the 92 strains from patients with uncomplicated infections, as contrasted with 15 (68%) of the 22 isolates from women with pelvic inflammatory disease ($P < 0.01$). The means of the MICs of penicillin G were $0.06 \mu\text{g/ml}$ for the former and $0.14 \mu\text{g/ml}$ for the latter.

Authors' summary

Non-specific genital infection

Early detection of chlamydial inclusions combining the use of cycloheximide-treated McCoy cells and immunofluorescence staining

B. J. THOMAS, R. T. EVANS,
G. R. HUTCHINSON, AND
D. TAYLOR-ROBINSON (1977).

Journal of Clinical Microbiology, 6,
285-292

Detection of *Chlamydia trachomatis* inclusions only 21 hours after a specimen reaches the laboratory has been achieved by the combined use of cycloheximide-treated McCoy cells and immunofluorescence staining. Moreover, cells exposed to cycloheximide were more sensitive for detecting chlamydial inclusions than those pretreated by irradiation, since larger numbers of inclusions were found in the former cells. The application of this rapid and sensitive method allows a diagnosis of chlamydial infection to be made before antibiotic therapy is started. In this way, it should enable the treatment of non-specific genital infections to be placed on a more rational basis.

Authors' summary

Survival of chlamydiae after cooling to -196°C

M. J. PRENTICE AND J. FARRANT (1977).
Journal of Clinical Microbiology, 6, 4-9

Factors influencing the survival of chlamydiae after freezing were re-examined. From the data presented, it is suggested that preservation of laboratory-grown chlamydiae is best achieved through the use of sucrose as the cryoprotective agent, in the presence of 10% serum. Dimethyl sulfoxide and glycerol are more toxic. The period of exposure to sucrose before freezing must be kept as short as possible and be at 4°C rather than at room temperature. The rate of cooling during freezing in sucrose is not important; however, cooling at a rate slower than 1°C/min should be avoided. Since chlamydial survival is increased by rapid thawing, the volume of the sample should be kept to a minimum. Thawed suspensions should be inoculated on to cell monolayers without delay. The application of these methods may increase the proportion of stored clinical specimens in which chlamydiae can be found.

Authors' summary

Modification of the microimmunofluorescence test to provide a routine serodiagnostic test for chlamydial infection

J. D. TREHARNE, S. DARUGAR, AND
B. R. JONES (1977).

Journal of Clinical Pathology, 30,
510-517

A modification of the microimmunofluorescence test to provide a practicable routine serodiagnostic test for detecting and characterising chlamydial infection is described which uses four antigen pools, one of which corresponds with each of the four main clinical and epidemiological types of chlamydial infection. The three subgroup A *Chlamydia* (*Chlamydia trachomatis*) pools are: pool 1, hyperendemic trachoma TRIC agent serotypes A, B, and C; pool 2, paratrachoma TRIC agent serotypes D, E, F, G, H, I, and K; pool 3, lymphogranuloma venereum (LGV) agent serotypes L1, L2, and L3. Pool 4 contained four representative isolates of subgroup B *Chlamydia* (*Chlamydia psittaci*).

For routine purposes sera need be screened only against these four representative antigen pools. This will detect antibody and indicate which clinical and epidemiological type of chlamydial infection is implicated, thereby clearly distinguishing those infections that are due to *C. psittaci*. The pattern of the

cross-reactions may indicate the individual serotype involved, and further titration requiring a maximum of four individual antigens is sufficient to determine the serotype. The slight loss in sensitivity (twofold) is more than compensated for by the reduction in cost and the tenfold increase in the total number of sera which can be examined.

Authors' summary

Treatment of patients suffering from chlamydial infections

E. M. C. DUNLOP (1977).

Journal of Antimicrobial Chemotherapy, 3, 377-380

Chlamydial, gonococcal, and herpes virus infections in neonates

G. W. CSONKA AND E. D. COUFALIK (1977).

Postgraduate Medical Journal, 53,
592-594

Chlamydial infections of the eye (Leading Article) (1977). *Lancet*, 2, 857-858

Morphology and ultrastructure of *Ureaplasma urealyticum* in agar growth

F. T. BLACK AND O. VINSTER (1977).

Acta pathologica et microbiologica Scandinavica, 85, 281-285

Effect of urea concentration on growth of *Ureaplasma urealyticum* (T-strain mycoplasma)

G. E. KENNY AND F. O. CARTWRIGHT (1977). *Journal of Bacteriology*, 132, 144-158

Immunological analysis of plasma membranes of a T-strain of mycoplasma (*Ureaplasma urealyticum*)

N. ROMANO, R. LA LICATA, AND
G. SCARLATE (1977).

Infection and Immunity, 16, 734

Trichomoniasis

The influence of antimetabolites on *Trichomonas vaginalis* as an experimental model

C. P. CHRISTOW (1976).

Microbios, 17, 87-92

Candidosis

Kinetics of phagocytosis and intracellular killing of *Candida albicans* by human granulocytes and monocytes

P. C. J. LEIJH, M. T. VAN DEN BARSELAAR, AND R. VAN FURTH (1977).
Infection and Immunity, 17, 313-324

Comparative susceptibility of *Candida albicans* to amphotericin B and amphotericin B methyl ester

R. M. BANNATYNE AND R. CHEUNG (1977).
Antimicrobial Agents and Chemotherapy, 12, 449-454

Genital herpes

Non-venereal sclerosing lymphangitis of the penis following herpes progenitalis

W. J. B. M. AN DE STAAK (1977).
British Journal of Dermatology, 96, 679-684

Genital herpes and Type 1 *Herpesvirus hominis*

T. W. CHANG (1977).
Journal of the American Medical Association, 238, 155

Techniques for typing *Herpesvirus hominis* antibody: a comparison of inhibition of peroxidase-labelled antibody staining with inhibition of indirect haemagglutination and with micro-neutralisation

G. GERNA AND R. W. CHAMBERS (1977).
Journal of Medical Microbiology, 10, 309-316

Some properties of recombinants between Type 1 and Type 2 herpes simplex viruses

I. W. HALLIBURTON, R. E. RANDALL, R. A. KILLINGTON, AND D. H. WATSON (1977). *Journal of General Virology*, 36, 471-484

Elisa for herpes simplex virus Type 2 antibodies (Letter)

P. C. GRAUBALLE AND B. F. VESTERGAARD (1977). *Lancet*, 2, 1038-1039

Radioimmunoassay of herpes simplex virus antibody. Correlation with ganglionic infection

B. FORGHANI, T. KLASSEN, AND J. R. BARINGER (1977). *Journal of General Virology*, 36, 371-376

Failure of levamisole in herpes simplex (Letter)

K. A. MEHR AND L. ALBANO (1977).
Lancet, 2, 773-774

Other sexually transmitted diseases

Venereal warts v. Bowen disease

A. LUPULESCU, A. H. MEHREGAN, H. RAHBARI, H. PINKUS, AND D. J. BIRMINGHAM (1977).
Journal of the American Medical Association, 237, 2520-2522

Verrucous growths of the genitalia in young patients are usually condylomata acuminata. In five cases this type of genital lesion, viewed by light microscopy, showed dysplastic cellular changes of Bowen disease, a diagnosis that was further confirmed by electron microscopy. Bowen disease should be included in the differential diagnosis of verrucous lesions of genitalia in young people and should be ruled out by histological examination.

Authors' summary

Condyloma acuminatum and squamous carcinoma of the vulva

R. M. RHATIGAN AND R. O. SAFFOS (1977).
Southern Medical Journal, 70, 591-594

The association of condyloma acuminatum and squamous carcinoma of the vulva in three cases, and condyloma followed by squamous dysplasia in a fourth, is reported. This association has previously been reported in both male and female genitalia and in the perineal area. In our four cases, cancer or squamous dysplasia followed long-standing or extensive condyloma, but there was no evidence of transformation from condyloma to carcinoma. In one of the cases, areas of squamous dysplasia occurred in multiple foci within a large condyloma. It is not known whether condyloma acuminatum is a precancerous skin lesion, but long-standing or extensive condylomas should alert the physician to the possibility of carcinoma.

Authors' summary

Molluscum contagiosum venereum in a women's outpatient clinic: a venereally transmitted disease

J. K. WILKIN (1977).
American Journal of Obstetrics and Gynecology, 128, 531-535

Molluscum contagiosum in a perigenital distribution was noted in 14 female patients who were attending a gynaecology/family planning clinic. The author examined the obstetric, marital, and sexual histories of these patients and concludes that the genital distribution, prevalence in the sexually active, documented association with sexually transmitted diseases such as gonorrhoea, and the presence of genital molluscum contagiosum in sexual partners indicate that this is a sexually transmitted disease.

(Although the author's conclusion may well be right, it should be mentioned that the results of contact tracing of patients with this disease are not very convincing. In the present study, two of the 14 patients claimed that their sexual partners had similar lesions, but neither of these seems to have been examined.)

J. D. Oriel

A case of chancroid

K. HARVEY, L. BISHOP, D. SILVER, AND T. JONES (1977).
Medical Journal of Australia, 1, 956

Miscellaneous

e Antigen among male homosexual patients

P. D. SIMMONS, M. N. ISLAM, S. KNOTT, J. E. BANATVALA, AND M. SUPRAN (1977).
British Medical Journal, 2, 1458

In this study from St Thomas's Hospital, London, hepatitis B surface antigen (HBsAg), e antigen and its antibody (anti-e), antibody to core antigen (anti-HBc) and the presence of Dane particles were determined in sera from a selected group of male patients.

Of 1602 patients tested 58 (3.6%) were HBsAg positive. Of 360 male homosexuals 20 (5.5%) were HBsAg positive, compared with 34 (3.4%) of 993 male heterosexuals. This difference was not significant ($P > 0.05$). However, e antigen was present in the sera of nine (45%) of 20 homosexuals, but in only four (13%) of 30

heterosexuals ($P < 0.02$). There was no significant difference in the proportion of HBsAg-positive homosexual and heterosexual patients who were anti-e positive or anti-HBc positive, and similar anti-HBc titres were present in both groups.

e Antigen is thought to be a marker of hepatitis-B virus infectivity, and the importance of care in handling sera from homosexuals is stressed. The Dane particle, which is probably the virus of hepatitis-B, was detected in 10 (77%) of 13 e-antigen positive sera but in only two (25%) of 8 which contained anti-e ($P < 0.02$).

J. D. Oriel

Corynebacterium vaginale in women with leucorrhoea

W. E. JOSEY, W. J. MCKENZIE, AND D. W. LAMBE (1977).

American Journal of Obstetrics and Gynecology, 126, 574-577

The authors studied 288 patients attending the Emory University Clinic, Atlanta, Ga, in whom leucorrhoea was found on examination. Specimens were taken from the vagina and cervix for culture, and in the majority direct microscopy was performed on wet preparations of the vaginal discharge. The patients were aged between 14 and 69 years. Cultures were also obtained from a control group of 30 women with no symptoms or signs of genital tract disease.

Cultures of *C. vaginale* were obtained from 164 (56.9%) of 288 patients with leucorrhoea, but from only four (13.3%) of 30 in the control group. A subgroup of 100 patients was studied to determine the probable cause of their leucorrhoea. In 13, the cause was non-infective. Among the other 87, yeasts were recovered from 54, *T. vaginalis* from 15, and *C. vaginale* from 33.

The authors discuss the pathogenicity of *C. vaginale*. They consider the characteristic discharge to be of a pasty consistency, clinging to the vaginal wall, and with a distinctive odour. They believe that cultural methods are the only reliable diagnostic techniques, and that 'blue cells' on a wet film are of less value. They use ampicillin or cephalixin for treatment.

(Further data on these patients can be found in Epidemiological characteristics of women infected with *Corynebacterium vaginale*, W. E. Josey and D. W. Lambe

(1976). *Journal of the American Venereal Disease Association*, 3, 9-13.)

G. D. Morrison

Evaluation of tetracycline or penicillin and ampicillin for treatment of acute pelvic inflammatory disease

F. G. CUNNINGHAM, J. C. HAUTH, J. D. STRONG, W. N. P. HERBERT, L. C. GILSTRAP, R. H. WILSON, AND S. S. KAPPUS (1977).

New England Journal of Medicine, 296, 1380-1383

To evaluate guidelines for outpatient treatment of acute pelvic inflammatory disease recommended by the Center for Disease Control we studied 197 afflicted women. They were treated either with tetracycline hydrochloride 1.5 g immediately followed by 500 mg six-hourly for 10 days or with aqueous procaine penicillin G, 4.8 megaunits intramuscularly, with 1 g probenecid by mouth, followed by ampicillin 500 mg six-hourly for 10 days. Of the whole group, 92% were subsequently seen at least once to assess efficacy of clinical and microbiological treatment.

N. gonorrhoeae was isolated from the lower genital tract in 68% of these women, and although they had a quicker symptomatic response than those with non-gonococcal infection ($P < 0.01$), the two regimens were equally effective in producing clinical cure. However, subsequent identification of a pelvic abscess was 10 times more common in women from whom *N. gonorrhoeae* was not isolated.

Therapy for pelvic inflammatory disease must be empirical, since it is impossible to distinguish clinically between gonococcal and non-gonococcal infection, and our data indicate that both regimens recommended by the Center for Disease Control are effective.

Authors' summary

Rosamicin—a new drug for the treatment of bacterial prostatitis

A. BAUMUELLER, U. HOYME, AND P. O. MADSEN (1977).

Antimicrobial Agents and Chemotherapy, 12, 240-242

Rosamicin, a new macrolide substance, was investigated in dogs and humans with regard to its usefulness for the treatment of bacterial prostatitis and compared with the well-known macrolide erythro-

mycin. In dogs with normal and experimentally infected prostates, concentration ratios for rosamicin in prostatic secretion, interstitial fluid (obtained from implanted tissue chambers), and tissue were significantly higher than for erythromycin. The difference was even more pronounced in human prostatic tissue, obtained by transurethral resection. With its broad spectrum against many common urinary pathogens, rosamicin seems to be a promising drug for the treatment of acute and chronic bacterial prostatitis.

Authors' summary

Rosamicin in urethral and vaginal secretions and tissues in dogs and rats

U. HOYME, A. BAUMUELLER, AND P. O. MADSEN (1977).

Antimicrobial Agents and Chemotherapy, 12, 237-239

In animal studies we investigated the distribution of rosamicin in plasma and in urethral and vaginal tissues in rats as well as in urethral and vaginal secretions in dogs. We found concentration ratios between urethral secretion and plasma of 1.9 and between vaginal secretion and plasma of 2.4. The rosamicin concentration in urethral and vaginal tissue significantly exceeded the levels of all other tissues investigated. Because rosamicin could be valuable for the treatment of bacterial urethritis and the colonisation of the vaginal introitus with faecal bacteria in women, it should be investigated clinically in this respect.

Authors' summary

In vitro activity of rosamicin against *Neisseria* and *Haemophilus*, including penicillinase-producing strains

C. C. SANDERS AND W. E. SANDERS (1977).

Antimicrobial Agents and Chemotherapy, 12, 293-294

Microbial flora of the vagina and cervix

C. M. CORBISHLEY (1977).

Journal of Clinical Pathology, 30, 745-748

The bacteriology of the cervix and uterus

R. A. SPARKS, B. G. A. PURRIER, P. J. WATT, AND M. ELSTEIN (1977).

British Journal of Obstetrics and Gynaecology, 84, 701-704

The interesting case. Fled drug eruption due to metronidazole

R. P. C. NAIK AND G. SINGH (1977).

Dermatologica, 155, 59-60

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Contents

Editorial: The nitroimidazole family of drugs *page 69*

Studies on strain sensitivity of *Trichomonas vaginalis* to metronidazole
J. G. MEINGASSNER, L. HAVELEC, AND H. MIETH (Austria) *page 72*

Secnidazole: a 5-nitroimidazole derivative with a long half-life
D. VIDEAU, G. NIEL, A. SIBOULET, AND F. CATALAN (France) *page 77*

Metronidazole and tinidazole in a single large dose for treating urogenital infections with *Trichomonas vaginalis* in men
N. KAWAMURA (Japan) *page 81*

Abnormal forms of *Trichomonas vaginalis*
J. JOHN AND S. SQUIRES (Gt Britain) *page 84*

Strain related infectivity of *Neisseria gonorrhoeae* for the guinea-pig subcutaneous chamber and the variability of the immune resistance in different breeds of guinea-pig
P. NOVOTNY, E. S. BROUGHTON, K. COWNLEY, M. HUGHES, AND W. H. TURNER (Gt Britain) *page 88*

Serological diagnosis of gonorrhoea by an enzyme-linked immunosorbent assay (Elisa)
A. A. GLYNN AND C. ISON (Gt Britain) *page 97*

The antimicrobial susceptibility of *Chlamydia trachomatis* in cell culture
G. L. RIDGWAY, J. M. OWEN, AND J. D. ORIEL (Gt Britain) *page 103*

Treatment and prognosis of non-specific genital infection
B. A. EVANS (Gt Britain) *page 107*

Postgonococcal urethritis: relationship to penicillin sensitivity of the gonococcus
G. R. KINGHORN (Gt Britain) *page 112*

Current approaches to the diagnosis of herpes genitalis
E. M. BELSEY AND M. W. ADLER (Gt Britain) *page 115*

Acquisition of Type 1 herpes simplex vulvitis within a monogamous relationship
J. E. WHITNEY, G. R. B. SKINNER, AND A. BUCHAN (Gt Britain) *page 121*

Default patterns of patients attending clinics for sexually transmitted diseases
J. D. H. MAHONY, J. BEVAN, AND B. WALL (Gt Britain) *page 124*

Evaluation of the early morning smear investigation
P. D. SIMMONS (Gt Britain) *page 128*

Asymptomatic gonococcal urethritis in a male transsexual female
N. J. FIUMARA AND S. ASVADI *page 130*

Abstracts *page 132*

28.9.78

June 1978 Vol. 54 No. 3

British Journal of
Venereal Diseases



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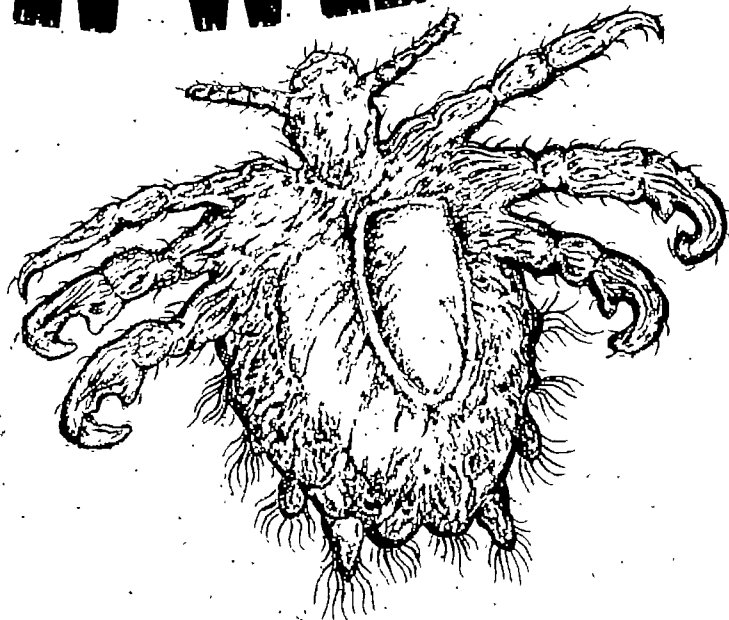
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Sexually transmitted diseases on a tropical island

V. S. RAJAN

From Middle Road Hospital, Singapore

Introduction

Singapore is a bustling cosmopolitan island city, situated at the southern tip of Peninsula Malaysia. It is 602 square kilometres with a population density of 3784 persons per square kilometre. The population is made up of Chinese (76%), Malays (15%), Indians (7%), and others (2%). The distribution of the sexes in the population is equal except for Indians who still exhibit a migrant pattern with a sex ratio of 1.4 males to 1 female. The population is young with 60% between the ages of 15 and 59 years.

The growing and expanding economy has provided full employment for residents and it has attracted migrant workers from the region. Singapore today enjoys an annual per capita income of \$5969 (US\$2436), second only, in Asia, to Japan.

In recent years, there has been a tendency for women to be economically independent, seeking employment in the industrial and commercial sectors. The proportion of economically active women between the ages of 15 and 44 years has risen from 21% in 1957 to 42% in 1975. Singapore is situated at the crossroads of international sea and air travel. In 1976, 1 492 218 tourists, businessmen, and officials visited the republic and stayed for about 3.3 days (Department of Statistics, 1977; Ministry of Health, 1977).

Epidemiology

Sexually transmitted diseases (STDs) have been notified only since 1 August 1977 under the Infectious Disease Ordinance. Hence, no national figures on the incidence of the different diseases are available except for those from public clinics. A survey showed that the true national incidence of syphilis and gonorrhoea was at least two and probably five times the reported incidence from public clinics (Morton,

1971). Nevertheless, the epidemiological trends are clear.

In 1950 the incidence of infectious syphilis was 160 per 100 000 population and gonorrhoea 325 per 100 000 population. In the early 1960s, these figures dropped to between 7 and 9 for syphilis and 125 for gonorrhoea; since then there has been a steady increase. In 1970, the incidence of gonorrhoea and infectious syphilis was 136 and 14 respectively per 100 000 population. In 1975, the rates were 21.4 for syphilis and 149 for gonorrhoea per 100 000 population. It is of interest to note that the number of cases of infectious syphilis dropped from 428 in 1975 to 201 in 1976. A further 40% decrease has been recorded for the first half of 1977. The gonorrhoea rates for 1976 have gone up but the redeeming feature has been the marked improvement in the identification rates in women. This has remained so for the first half of 1977 and, surprisingly, the number of infections of gonorrhoea in women has outstripped that in men by 3:1. A 20% drop in infections in men has been recorded for the first half of 1977. In 1976, 1627 cases of chancroid, 88 cases of lymphogranuloma venereum, 255 cases of genital herpes, 461 cases of non-specific urethritis, and 559 cases of venereal warts were reported. Singapore is one of the few countries that has reported high figures for chancroid and lymphogranuloma venereum.

The age groups affected by STDs are mainly between 20 and 24 years (30%), and between 25 and 29 years (21%). In men, 18.5% are between the ages of 15 and 19 years but only 5.6% of women are in this age group. Thus promiscuity in the female appears to begin later than is witnessed in the West. Few cases of acquired STDs are seen in either boys or girls under the age of 18 years. The index case in 60% of patients is a female prostitute; 13.5% of patients are infected overseas. Homosexuality is not a problem here (Middle Road Hospital, 1950-76). A study of prostitutes showed that 46% have syphilis, 8.5% gonorrhoea, 14.5% asymptomatic chancroid, and 64% possibly have asymptomatic or old chlamydial infections. This same study showed that only 5% were free of any STD (Khoo *et al.*, 1977).

Address for reprints: Dr V. S. Rajan, Middle Road Hospital, Singapore

Received for publication 19 November 1977

Control aspects

The epidemiological data show that health education and control are very important if the incidence of infection is to be reduced in the biggest group at risk. As there is little infection in boys who are under the age of 18 years and in girls under 20 years, health education programmes are aimed at reaching the general public, children in their final years at school, and boys doing National Service. An island-wide national health campaign against six infectious diseases including STDs was launched in 1976, followed by a booster campaign on STDs alone in September 1977. Pre- and post-campaign assessment showed a statistically significant increase in knowledge of STDs in secondary schoolchildren and in uniformed personnel in the armed forces. The campaign's impact on change of attitudes is being assessed.

Prostitutes (male and female) are the largest reservoir of infection. Although their existence has no legal sanction, their role in the spread of STDs and the need to control disease in this group of people is recognised. Hence a Medical Scheme incorporating the assistance and functions of the police department, computer services department, laboratory, clinical services, and general practitioners participating in the scheme, was launched in August 1976. The philosophy of the scheme has been one of persuasion and the use of subtle legal pressures to ensure its success. In the brothels today, 85% of the prostitutes in the scheme are readily accessible for periodic checks and treatment when found to be infected. The dramatic reduction in infectious syphilis during the past two years, the 20% drop in the rate of gonorrhoea in men during the first half of 1977, and the 3:1 female to male ratio for the incidence of gonorrhoea can be attributed to the success of this scheme.

We have adopted the following policy on epidemiological treatment. All contacts are investigated but are not routinely treated. However, if epidemiological evidence strongly suggests an infection, the contact is treated after relevant tests have been performed. In the Medical Scheme, only those found to be infected are treated and no routine epidemiological treatment is offered.

Doctors and laboratories are now obliged to notify cases of gonorrhoea, non-specific urethritis, infectious and non-infectious syphilis, chancroid, and also cases of genital discharge and ulcers not specifically diagnosed but considered to be of venereal origin. The notification is on a non-nominal basis but the patient's age and sex has to be given. Practitioners are encouraged to provide information on the patient's sexual contacts to the

Sexually Transmitted Diseases Epidemiological Control Unit if their patient has no objection. In a country where patients frequently change their doctors, over-reporting is possible.

Singapore was one of the first countries in the world to report β -lactamase producing strains of gonococci. During the past 15 months only 20 strains have been isolated out of more than 7000 tested. The Central Neisseria Laboratory routinely tests all isolates for penicillin resistance. When detected the patient and all sex contacts are immediately traced and treated. We favour the use of kanamycin for treating β -lactamase strains, and we have had no failures with a single 2 g injection.

Treatment facilities

Patients with STDs have the choice either of seeking treatment from a general practitioner or attending a public clinic run by the hospital division of the Ministry of Health. The speciality is combined with dermatology and a teaching hospital is the national centre for the combined speciality and epidemiological control. Treatment of STDs is free. Treatment and laboratory facilities are of good standard. The national laboratory is well equipped with facilities to perform 800 VDRL (Venereal Disease Research Laboratory) tests, 50 FTA/ABS (fluorescent treponemal antibody absorption) tests, and 1000 gonococcal cultures a day. Facilities to isolate mycoplasma and *Chlamydia* are available, and cultures for *Haemophilus ducreyi* are also routinely performed. The standard of clinical practice in the private sector leaves something to be desired. A recent survey showed that gonorrhoea is mostly diagnosed on clinical grounds, although serological tests for syphilis are more frequently requested in patients with genital ulcers. About six private laboratories have facilities to perform serological tests and gonococcal cultures.

For the treatment of syphilis, the long acting benzathine penicillin is recommended. For gonorrhoea, we favour an 'instant treatment' schedule and either 3 megaunits of injection procaine penicillin or 2 g of ampicillin with probenecid is the standard treatment. Fifty-two per cent of local strains of gonococci are less susceptible to penicillin but with this dosage the treatment failure rate is less than 4%. As second line drugs—and for β -lactamase producing strains—injection kanamycin 2 g, injection kanandomycin 1.2 g, or injection spectinomycin 2 g is recommended. The same dosage is used for men and women. In our experience against local strains, all bacteriostatic antibiotics—such as the tetracyclines used in instant treatment regimens—have given poor results and are not recommended.

For chancroid, 7 to 10 days of tetracycline 1 g daily or streptomycin 1 g daily is routinely recommended. Better results are obtained with streptomycin. Clinical trials indicate that co-trimoxazole is a promising alternative. For lymphogranuloma venereum and non-specific urethritis, tetracyclines for two or three weeks are recommended.

In all patients with infectious syphilis and gonorrhoea, attempts are made to trace sex contacts. On the average we have a 30% success rate.

Teaching

There is no full-time department of dermatovenereology in the university. Government specialists are employed, part-time, to teach this subject. Undergraduates receive 96 hours of teaching in the combined speciality during their fourth and final years of training. The whole spectrum of STDs is taught and students are encouraged to take specimens for darkground examination and smears, and to examine the slides. Due emphasis is given to epidemiology and control.

In the final year the student is obliged to sit for an assessment test in the speciality. Questions on STDs are included in the final year multiple choice paper in medicine.

Candidates doing postgraduate courses in medicine, obstetrics, and gynaecology attend a two-hour session in venereology. For general practitioners occasional courses are held in conjunction with the College of General Practitioners. Standard recommendations and treatment guidelines are published periodically in the *Epidemiological News Bulletin* published by the Ministry of Health and distributed free of charge to all registered medical practitioners in the republic.

STD is not a neglected discipline. The government has been concerned at the way it has spread and has given support to control it. It has been the national policy to share our advanced facilities and expertise with neighbouring countries so that there is international participation and co-operation in curbing this modern plague.

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Cell-mediated immunity during syphilis

A review

CHARLES S. PAVIA, JAMES D. FOLDS, AND JOEL B. BASEMAN

From the Department of Bacteriology and Immunology, School of Medicine, University of North Carolina, North Carolina 27514, USA

SUMMARY Evidence is presented which reinforces the complexity of the host-parasite interaction during the course of syphilis. Infection with *Treponema pallidum* evokes a complicated antibody response and an assortment of cell-mediated immune reactions in the host. It appears that humoral immunity plays a minor role towards the complete elimination of syphilitic infection while the cellular limb of the immune response may be an important host defence mechanism. Information now available indicates that a state of anergy, or immunosuppression, exists in the early stages of human and experimental rabbit syphilis based upon negative skin reactions to *T. pallidum* antigen(s), the abnormal histological appearance of lymphoid organs, and impaired *in vitro* lymphocyte reactivity. It is also evident that in the later stages of the disease cellular immunity becomes activated as delayed type skin reactions can normally be elicited in tertiary syphilitics and lymphocyte behaviour in cell culture appears normal. Several mechanisms have been invoked to explain the delay in an effective immune response against syphilitic infection and the duration of the disease: (1) a capsule-like substance on the outer surface of virulent *T. pallidum* may act as a barrier against treponemocidal antibody; (2) this material and other biological properties of virulent treponemes could enable spirochaetes to escape being engulfed by macrophages and other phagocytic cells; (3) antigenic competition among different treponemal antigens causing partial tolerance; (4) *T. pallidum* infection may bring about the elaboration of immunosuppressive substances of host or treponemal origin which inhibit the proper function of lymphocytes, macrophages, and other cell types.

Introduction

Since Schaudinn and Hoffman (1905) identified *Treponema pallidum* as the causative agent of syphilis, the immunological phenomena associated with the disease have intrigued researchers and clinicians. During the past seven decades attempts to grow virulent *T. pallidum* on artificial media and to develop a successful vaccine have proved fruitless although there have been several unconfirmed reports claiming successful *in vitro* cultivation of the pathogenic spirochaete (Noguchi, 1911; Jones *et al.*, 1976). Without cultivable virulent treponemes, pure organisms devoid of host tissues are not available in

the quantities needed for biological and immunological studies. Consequently the nature of the basic immunological mechanisms elicited in the diseased host by the fastidious spirochaete or its fractions remains an enigma. Despite these obstacles considerable information has accumulated concerning antibody formation, cell-mediated immune reactions, and other aspects of host resistance to syphilitic infection (Turner and Hollander, 1957; Cannefax, 1965; Wigfield, 1965).

ANTIBODY FORMATION

Infection with *T. pallidum* stimulates the host defence mechanisms and provokes a complex antibody response. Data strongly indicate that at least two varieties of antibody are produced as syphilis progresses from early to late stages. One type (Wassermann or reagin) is non-specific and reacts primarily with tissue extracts composed of lipids; the other type (immobilising, agglutinating, fluorescent, treponemal) is specific since it reacts with some,

This work was supported by PHS grants 1-R01-GM-21313 from the National Institute of General Medical Sciences and AI-11283 from the National Institute of Allergy and Infectious Diseases

Address for reprints: Charles S. Pavia PhD, 494 HSW, Department of Laboratory Medicine, University of California, San Francisco, California 94143, USA

Received for publication 17 October 1977

as yet, unidentified component(s) of the treponeme. It has been reported that one or more of these antibodies are treponemocidal and are supposedly protective (Nelson and Meyer, 1949). However the role of humoral immunity has not been delineated as some individuals with syphilis progress through primary, secondary, and tertiary stages of infection despite the formation of these various antibodies. Furthermore attempts to demonstrate a humoral mechanism by *in vivo* passive immunisation experiments with presumably immune syphilitic sera from *T. pallidum* infected rabbits have had limited success (Turner *et al.*, 1973; Weiser *et al.*, 1976) thereby suggesting that antitreponemal antibodies provide, at best, partial protection against syphilitic infection.

CELL-MEDIATED IMMUNITY

The part played by cell-mediated immunity (CMI) in the host defence against *T. pallidum* infection has also not been determined. Before the work of the past 10 years on the possible role of cellular immunity in syphilis, very little information was available on this aspect of the host response. In the early part of this century Noguchi (1911, 1912) was able to obtain a skin reaction using an extract of pure cultures of *T. pallidum* which was termed luetin. He found that an intradermal injection of luetin after repeated injections of either living or dead *T. pallidum* into rabbit testes elicited a local inflammatory reaction, and yet there was no reaction in rabbits suffering from acute orchitis. He also demonstrated similar skin reactions (sometimes severe) in tertiary stages of human syphilis, while those patients in the primary and secondary stages exhibited occasional reactions or mild ones. In contrast to Noguchi's findings, Rich *et al.* (1933) were unable to demonstrate any allergic type reactions in previously infected rabbits when reinoculated with virulent *T. pallidum* at periods ranging from 28 to 482 days after the initial immunising infection. These authors concluded that allergic inflammation may not be associated with any acquired immunity in experimental rabbit syphilis. In another report, Marshak and Rothman (1951) found that only patients who had acquired gummatous or congenital syphilis gave strong tuberculin-type reactions to a purified suspension of *T. pallidum* prepared from infected rabbit testes, while six of seven individuals with secondary acquired syphilis yielded negative reactions. Similarly only 60% of rabbits infected for a two- to 12-month period gave a positive skin reaction to the treponemal extract. No correlation existed between the duration of the infection and the degree of skin reaction. An analogous study conducted by a French group (Tlifolet *et al.*, 1955) showed that delayed-type skin

reactions to dead *T. pallidum* were rarely elicited in patients with primary and secondary syphilis, whereas a large percentage of tertiary syphilitics reacted positively to intradermal injection of this treponemal extract. Other experiments performed by Laird and Thorburn (1966) showed that 67% of their patients with tertiary syphilis gave delayed-type hypersensitivity reaction to an extract of *T. pallidum*, the luotest, which occurred after repeated challenge. The unresponsiveness in early syphilis seems to be specific to *T. pallidum* antigens since skin reactions to several non-treponemal antigens—such as, mumps, old tuberculin, and varidase—were reported in patients at various stages of syphilis (Wright and Grimble, 1974).

Some investigations have been performed relating the histological appearance of lymphoid tissues with the presence of *T. pallidum* infection. In a study in which lymph nodes of 20 patients with early syphilis were removed, Turner and Wright (1973) were able to show a depletion of lymphocytes in the paracortical areas of these tissues. Numerous treponemes were found in these areas using the silver impregnation technique. Levene *et al.* (1971) while conducting a study of infants dying from congenital syphilis discovered a diminution of lymphocytes in the area around the central arteriole of the spleen. Comparable results were obtained by Festenstein *et al.* (1967) who infected neonatal rabbits with *T. pallidum*. These infected animals exhibited a runting syndrome in which death usually occurred within three months. Depletion of lymphocytes in the white pulp areas of the spleens of these rabbits was found although treponemal invasion of this organ was not observed. The paracortical areas of lymph nodes and the area around the central splenic artery are thymus-dependent areas involved in mediating cellular immune reactions of the host, and defective CMI is associated with lymphocyte depletion in these areas (Parrott *et al.*, 1966). From these studies on the structural appearance of lymphoid tissue it is apparent that thymus-dependent (T-cell) areas are adversely affected during syphilitic infection.

Another parameter used to examine the cellular mechanisms involved in *T. pallidum* infection has been the attempt by several investigators to activate the reticuloendothelial system by inoculating rabbits with BCG* organisms and then monitoring what effect this treatment had on the course of experimental disease (Graves and Johnson, 1975; Schell *et al.*, 1975b). In these studies prior injection with BCG was unsuccessful in preventing the develop-

*BCG is a viable attenuated strain of *Mycobacterium bovis* which in the form of a vaccine has been able to confer protection against mycobacterial infection and to stimulate non-specifically CMI in certain experimental models.

ment of syphilitic lesions in rabbits subsequently inoculated with virulent *T. pallidum*. Although there was a delay in the onset of lesions when immune treponemal serum was administered simultaneously with BCG, no evidence was found suggesting a synergistic protective effect between the activated macrophages and the antitreponemal antibodies. Despite these results whereby enhanced resistance to treponemal infection owing to presumably armed macrophages was not detected in BCG-infected animals, Schell and Musher (1974) have presented indirect evidence favouring the suggestion that infection with *T. pallidum* stimulates CMI. Basically they followed the model established by Blanden *et al.* (1969) in which infection by one organism confers resistance to infection with an antigenically unrelated organism. Rabbits were infected first with virulent *T. pallidum* and then challenged with *Listeria monocytogenes*. Resistance to *Listeria* infection was then evaluated weekly by measuring the number of organisms found in the spleens and livers of the infected animals. Enhanced ability to suppress the growth of *Listeria* was detected in the rabbits' livers between the third and sixth week after intravenous infection with virulent *T. pallidum*. However there was no indication that treponemes were engulfed by these activated phagocytic cells. Additionally, in a manner similar to the adoptive transfer model of Mackaness and Hill (1969), spleen cells from syphilitic rabbits together with *T. pallidum* were transferred to normal rabbits (Schell *et al.*, 1975a). A significant degree of resistance to subsequent challenge inoculation with *Listeria* was produced in the recipient animals. The results were based on the number of *Listeria* in the livers and spleens of these rabbits. When the spleen cells from the infected donor rabbits were first reacted with a highly specific antirabbit thymus serum and complement, Schell *et al.* (1975a) showed that transfer of immunity was abrogated indicating that thymus-dependent immunity was responsible for conferring resistance to *Listeria* infection. In a similar fashion Metzger and Smogor (1975) demonstrated partial immunity to *T. pallidum* infection by adoptive transfer of lymph node lymphocytes from syphilitic rabbits to normal ones. These investigators showed that a reduced number or a delayed incubation or absence of lesions occurred in the recipient animals after intradermal challenge with virulent *T. pallidum*. However since outbred animals were used in these experiments partial protection could have resulted from non-specific sensitisation occurring when allogeneic cells present in the putative immunocompetent lymphocyte population were transferred from infected to normal rabbits.

In vitro tests for CMI, notably lymphocyte trans-

formation and cell migration inhibition, have been used to monitor the cellular immune capability of man and animals to various diseases (Kantor, 1975; Rocklin, 1976) including syphilis (Badanoui *et al.*, 1969; Levene *et al.*, 1969; Janot *et al.*, 1971; Fulford and Brostoff, 1972; Musher *et al.*, 1974; Friedman and Turk, 1975; Musher *et al.*, 1975; Wicher and Wicher, 1975; From *et al.*, 1976; Pavia *et al.*, 1976, 1977a). In the lymphocyte transformation assay lymphoid cells usually derived from peripheral blood are cultured with a suitable stimulant, especially a mitogen—such as, phytohaemagglutinin (PHA), or concanavalin A (Con A), or a specific test antigen—in this case a treponemal preparation. The mitogen non-specifically induces a large number of cells to enlarge, proliferate, and undergo DNA synthesis whereas a narrower range of cells experience similar changes in a specific response to the antigen. The degree of blastogenesis is measured either by microscopical examination of the blast cells or more accurately by incorporating a radioactively labelled DNA precursor into newly synthesised DNA. The amount of label incorporated and therefore the rate of DNA synthesis is determined usually by scintillation counting which gives a quantitative assessment of lymphocyte responsiveness and presumably functional capacity. The results of this test using lymphocytes from syphilitic patients have so far been inconsistent. Several European investigators have been able to transform cells from individuals with syphilis. Badanoui *et al.* (1969) showed that positive lymphocyte responses to PHA and an antigen composed of *T. pallidum* (Nichols strain) can regularly be elicited from patients with syphilis at various stages of infection. In another study, Janot *et al.* (1971) using a saline suspension of the Nichols strain of *T. pallidum* as stimulant, demonstrated increased numbers of lymphoblastoid-like cells based upon the morphology of the lymphocyte populations in four of six patients with primary, nine of 11 with secondary, five of six with congenital syphilis, as well as all of 18 with cardiovascular or neurosyphilis, 30 with latent, and 12 patients with treated syphilis. Likewise Friedman and Turk (1975) reported elevated blastogenic responses to a *T. pallidum* preparation in patients with seropositive primary infection and those in the papular stage of secondary syphilis, although patients in the macular stage of disease gave poor responses. In addition individuals undergoing treatment with drugs exhibited marked lymphocyte reactivity to the *T. pallidum* antigen. Data contrary to these findings have been provided by other investigators. Levene *et al.* (1969) demonstrated that patients with primary and secondary syphilis had an impaired lymphocyte response to PHA. At the

same time plasma from patients with secondary syphilis was found to cause reduced levels of blastogenesis of normal lymphocytes. Other workers (Kantor, 1975; From *et al.*, 1976) have shown that serum or plasma from diseased patients hampered the PHA or Con A induced response of normal lymphocytes. In two studies Musher *et al.* (1974, 1975) showed that primary and secondary syphilitics exhibited a suppressed *in vitro* lymphocyte response to treponemal antigens derived from *Treponema refringens*, *Treponema phagedenis* biotype Reiter, and *T. pallidum*, and to non-treponemal antigens such as *Candida* and trichophytins. However these patients demonstrated a normal lymphocyte response to PHA, pokeweed mitogen, and streptolysin O. Moreover syphilitic serum possessed no *in vitro* inhibitory activity. Another *in vitro* correlate of CMI, the leucocyte migration inhibition test, has been used in several studies for examining the cellular response of syphilitic patients (Fulford and Brostoff, 1972; From *et al.*, 1976) and of experimentally infected rabbits (Wicher and Wicher, 1975; Metzger *et al.*, 1977). In this particular assay migration inhibition is measured directly using peripheral blood leucocytes or indirectly using sensitised lymphocytes and guinea-pig macrophages and placing them either in capillary tubes or in agar in the presence or absence of appropriate antigen. Cell migration is measured between 24 and 48 hours later and the relative areas of migration are determined, usually by planimetry, and the amount of inhibition of cell migration is calculated. Inhibition of cell migration is closely associated with the presence of *in vivo* cellular hypersensitivity of the host to that antigen (Rocklin, 1976). Using this assay Fulford and Brostoff (1972) assessed the reaction of 47 patients who were at various stages of syphilis to a commercial preparation of Reiter protein as antigen. They found that in 19 cases of primary syphilis, patients exhibited stimulation of migration; 24 patients with either secondary or latent syphilis showed neither stimulation nor inhibition. Only in those individuals with late active syphilis was there clear evidence of inhibition of migration which could be interpreted as a manifestation of CMI to Reiter protein. In a similar report (From *et al.*, 1976), it was demonstrated that increased cellular reactivity evaluated by migration inhibition in response to a *T. pallidum* antigen became evident after syphilitic patients received drug treatment. Another study (Wicher and Wicher, 1975) investigated the cellular response of syphilitic rabbits by measuring leucocyte migration in agarose using a *T. pallidum* antigen, a commercial Reiter antigen, and cardiolipin reagent as stimulants. A bimodal response was demonstrated by cells in the presence of each of the

commercial preparations and a low concentration of *T. pallidum* antigen. During the first four weeks of syphilitic infection, stimulation of leucocyte migration occurred (indicative of weak CMI), while after this period inhibition of migration was observed (indicative of strong CMI). When cells were exposed to a higher concentration of *T. pallidum* antigen inhibition of migration was seen from the first week of infection onwards. Similar data have been given by Metzger *et al.* (1977) in a long-term study whereby the macrophage migration inhibition test was used for evaluating the cellular response during experimental disease. It was demonstrated that from one month after infection lymphocytes from *T. pallidum* infected rabbits exposed *in vitro* to a sonicated treponemal suspension significantly inhibited the migration of guinea-pig macrophages. The inhibitory effect was maintained for at least two years of infection except for a transient decrease in inhibition activity between the fourth and sixth month.

In our series of experiments we investigated the CMI response of rabbits infected with *T. pallidum* by employing the lymphocyte transformation assay (Pavia *et al.*, 1976, 1977a) and the macrophage migration inhibition test (Pavia *et al.*, 1977b). Although the rabbit may not be suitable for certain immunological studies we chose this animal for our investigation as experimental infection in the rabbit has so far been the model of choice in attempting to elucidate the nature of immunological phenomena associated with the disease and for making comparisons with human syphilis. Furthermore a local lesion can be readily produced at the site of inoculation of virulent *T. pallidum* and the clinical course of the disease can easily be monitored as the infection progresses from early to late stages. The study consisted of collecting blood weekly from *T. pallidum* infected rabbits and culturing their peripheral blood lymphocytes with various mitogens and Reiter treponemal antigen. During the first three or four weeks of infection the transformation of lymphocytes from infected rabbits by Con A, PHA, and pokeweed mitogen was suppressed when compared with the lymphocyte response of non-infected control rabbits. In addition, serum from the donor rabbit significantly reduced the Con A-induced response of autologous lymphocytes. After four weeks, normal levels of blastogenesis were usually observed. When lymphocytes from these same infected animals were exposed *in vitro* to antirabbit immunoglobulin and to a Reiter treponemal preparation a different pattern emerged. Throughout the duration of infection normal levels of blast transformation were obtained in response to these stimulants.

In the next sequence of experiments peritoneal

exudate cells from *T. pallidum* infected rabbits were collected every two weeks and examined for migration inhibition activity in the presence of *T. phagedenis* biotype Reiter. Between five and 15 weeks after infection there was a significant inhibition of macrophage migration in response to three different concentrations of the Reiter treponeme. Before this period little or no inhibition had been evident. The inhibitory effect manifested in these animals could be interpreted as a response by sensitised cells to antigenic determinants of the Reiter treponeme which are shared with the virulent Nichols *T. pallidum* (Meyer and Hunter, 1967).

Our findings suggested an interrelationship between the appearance and regression of symptomatic infection and the *in vitro* response to T-cell mitogens (Con A, PHA, pokeweed mitogen) in the transformation test and *T. phagedenis* biotype Reiter in the migration inhibition assay. During the first few weeks of infection in which widespread dissemination of organisms occurred and severe ulcerative lesions developed the rabbits manifested poor lymphocyte reactivity, whereas during remission when lesions were subsiding (four to six weeks after infection) blastogenesis and migration inhibition markedly increased. This may reflect heightened T-cell reactivity at this stage of infection. Furthermore the normal blastogenic response to anti-immunoglobulins (putative B-cell mitogens) may correlate with the initiation of antibody synthesis to treponemal antigens and thus reflect an intact humoral expression of immunity in syphilis.

Recently we have reinforced previous data describing the apparent selectivity of the lymphocyte response during experimental *T. pallidum* infection by separating rabbit lymphocytes into T- and B-cell populations and monitoring their *in vitro* response to appropriate mitogens and *T. pallidum* antigen(s) (Pavia *et al.*, 1977c). It was clear that Con A and PHA-induced proliferation of the purified T cells was markedly reduced during early infection while the response to classical B-cell mitogens was normal. In striking contrast, the same enriched T-cell population from infected animals was stimulated by *T. pallidum* antigen(s). However autologous syphilitic serum significantly diminished the *in vitro* stimulatory effects of *T. pallidum* antigen(s) on the enriched T cells. A relevant conclusion from these studies is that during early disease the T cells that exist in the infected host respond poorly to certain mitogens while these cells, or a subpopulation of them, are readily stimulated by *T. pallidum*. This latter phenomenon, in turn, is subject to the regulatory effects of syphilitic serum which could adversely affect *in vivo* as well as *in vitro* lymphocyte reactivity.

Proposed mechanisms of immunity in syphilis

Limited information is available concerning the nature of immune mechanisms during *T. pallidum* infection. Although a role for humoral immunity was implicated when it was apparently demonstrated that protective antibodies directed against *T. pallidum* were present in serum taken from infected patients and rabbits (Turner, 1939; Turner *et al.*, 1948), it was later suggested (Turner and Hollander, 1957) that the phenomena probably represented an *in vitro* effect rather than a direct *in vivo* consequence. Also the general pattern of evidence indicates little correlation between the level of antibody and the degree of immunity to challenge of previously infected or immunised rabbits (Magnuson *et al.*, 1951; Metzger *et al.*, 1969; Miller, 1973) and it has also been demonstrated that complete protection against experimental *T. pallidum* infection can be accomplished without the immediate presence of circulating antibody (Miller, 1973).

A relationship may exist between the inherent properties of the infectious treponeme and the role of immune mechanisms in syphilis. Factors intrinsic to the treponeme may enable it to circumvent or block immunological attack. It has recently been shown (Zeigler *et al.*, 1976) that the virulent Nichols strain of *T. pallidum* possesses an extracellular layer. This outer coat was observed *in vivo* and *in vitro* after the organisms were exposed to ruthenium red. Before this report, only presumptive evidence for this structural component existed (Metzger *et al.*, 1961; Christiansen, 1963). Some researchers (Metzger *et al.*, 1961; Metzger, 1962; Miller, 1967) have advanced the hypothesis that acquired resistance may depend on the association between treponemidal antibody and this outer capsule-like material. It has been suggested that treponemidal antibody can exert an inhibitory effect on the organisms once the outer capsule has been removed or somehow been stripped off. What mechanisms are involved in the disappearance of this outer coat are not known but the relatively slow development of immunity to *T. pallidum* infection may be directly related to the poor antigenic nature of this outer material and its half life *in vivo*.

Since the role played by antibody towards the eradication of syphilitic infection is not clear it may be necessary to invoke cell-mediated immune phenomena as important factors in the infectious process. It has already been demonstrated (Turner and Hollander, 1957) that vaccines consisting of dead or purified antigen preparations of *T. pallidum* have generally been unsuccessful in providing effective protection against treponemal infection despite the formation of an abundance of antibody. However

complete resistance to *T. pallidum* infection has been produced by prior injections of motile virulent treponemes attenuated by γ -irradiation (Miller, 1973). Elberg (1973) showed that efficient induction of CMI in certain microbial infections requires living vaccines, whereas killed vaccines or purified bacterial antigens are less efficient forms of immunoprophylaxis. Taken together, these facts suggest that protection against *T. pallidum* infection may depend on a specifically activated cellular immune system. In addition it is becoming apparent that the cellular limb of the immune response is defective in the highly infectious early stages of syphilis and is mobilised at other stages. Antigenic competition has been considered a possible candidate underlying the hyporeactive state in early disease (Wright and Grimble, 1974). If *T. pallidum* possesses multiple antigenic determinants (and this seems likely) one antigen could selectively compete and interfere with the response to other antigens, thereby creating a condition of partial or split tolerance. Another conceivable explanation is that this transient period of immunosuppression may be the result of a modification of T-cell or macrophage function by blood factors or by other cellular components of host (immune complexes, suppressor substances) or treponemal (outer envelope, metabolic byproduct) origin generated during syphilitic infection. Effector lymphocytes may be suppressed by a 'blocking factor' analogous to that described in tumour immunity and in certain experimental systems (Hellstrom and Hellstrom, 1970) so that they are unable to differentiate or carry out effector function. In fact, inhibitory plasma or serum products have been demonstrated in syphilitic patients (Levene *et al.*, 1969; Kantor, 1975) and in *T. pallidum* infected rabbits (Pavia *et al.*, 1976; 1977c). In human syphilitic glomerulonephritis deposits of treponemal antigen-antitreponemal antibody complexes have been shown to contribute to tissue injury (Gambol and Reardon, 1975). Although suppressor cells have not been studied in syphilis they could be an important consequence of *T. pallidum* infection especially in the light of the recent finding of suppressor thymus-derived lymphocytes in patients infected with a variety of fungal organisms (Stobo *et al.*, 1976). In our recent report (Pavia *et al.*, 1977c) on experimental *T. pallidum* infection in rabbits the existence of suppressor-like function is suggested by a delay in the response to *T. pallidum* antigen(s) of unfractionated lymphocytes in contrast to purified cells. Suppressor cells could inactivate immune lymphocytes which arise at foci of treponemal infection. A regulatory effect exerted on immunocompetent cells by any of the preceding conditions could also block the release of soluble mediators

collectively known as lymphokines, one of which, migration inhibitory factor, encourages circulating blood monocytes to localise at sites of microbial invasion. The inability of effector cells such as lymphocytes and macrophages to be recruited to specific tissues in the diseased host and/or the capacity of *T. pallidum* to resist phagocytosis due to the organism's innate biological properties may delay the early destruction of virulent treponemes and contribute to the widespread dissemination and establishment of spirochaetes in selected host sites. Then as the infection progresses to later stages a transition in the immune status of the host takes place. Normal T-lymphocyte function is restored leading to an effective cell-mediated response which could allow host defence mechanisms to participate in treponemicidal activities. From the sequence just presented it should be realised that cell-mediated phenomena in syphilis are by no means expressions of a single mechanism. Different cell categories (T-cells, B-cells, monocytes, macrophages, neutrophils) may be involved and their relative contribution to immunity may depend on the dynamic interaction of various factors induced by *T. pallidum* infection. If indeed cell-mediated immunity is an important element against syphilitic infection then investigations should be carried out in order to determine the extent of its protective role in this disease.

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TPHA test

Experience at the Clinic of Dermatology, University of Milan

E. ALESSI AND L. SCIOCCATI

From the Clinic of Dermatology of the University of Milan

SUMMARY The results of serological investigations with the treponemal haemagglutination (TPHA) test are reported in a large number of luetic and non-luetic patients. From these the following conclusions can be drawn: (1) The sensitivity of the TPHA test is decreased if the test is carried out with micromethods; however, in our opinion, the same degree of sensitivity can be obtained either with the macromethod at dilution 1/80-1/60 or with micromethods at 1/20-1/40. (2) The sensitivity of this test is high, being similar to that of the fluorescent treponemal antibody absorption (FTA-ABS) test in primary and secondary syphilis and even higher than that of the FTA-ABS test in treated subjects. (3) The specificity of the reaction is high, as demonstrated by examining sera in patients with a negative history and clinical examination together with negative results to *Treponema pallidum* immobilisation (TPI) and FTA-ABS tests, and by studying biological false positive sera. (4) For the serological screening, it may be sufficient to perform the TPHA test with the 1/20-1/40 micromethod together with the Venereal Disease Research Laboratory (VDRL) test. In patients with suspected syphilis, it is advisable to perform the TPHA test by the macromethod, in combination with the FTA-ABS test.

Introduction

The treponemal haemagglutination (TPHA) test has been studied for several years at our institute and it is now used routinely for detecting anti-treponemal antibodies in patients attending the Centro Antivenereo (Center for Venereal Diseases) of the University of Milan.

The characteristics of this reaction are well known (Rathlev, 1967; Tringali, 1970; Coffey *et al.*, 1972; Garner *et al.*, 1972; Johnston, 1972; Luger and Spendlingwimmer, 1973; Young *et al.*, 1974; O'Neill, 1976). We should like to add our contribution, based on personal experience. Technical details are given in previous papers (Alessi *et al.*, 1973; Alessi and Scioccati, 1977).

Methods

The manual macromethod traditionally used for the TPHA test has recently started to be replaced

by micromethods because of the high cost of reagents and the need to automate the reaction in mass serological screening.

However, the data indirectly show that the sensitivity of the reaction is adversely affected by these modifications. Indeed, while those who use macromethods (Rathlev, 1967; Tringali, 1970; Luger and Spendlingwimmer, 1973) report reactivity early in the course of infection (as with the fluorescent treponemal antibody absorption (FTA-ABS) test), those who use micromethods (Coffey *et al.*, 1972; Garner *et al.*, 1972; Johnston, 1972; Young *et al.*, 1974) observe delayed reactivity, which in some cases occurs only at the beginning of the secondary stage.

These different findings can be accounted for by comparing the results of macromethods with those of micromethods on 479 sera* (Alessi and Scioccati, 1977) (Tables 1 and 2). Our investigation showed that with the same serum dilutions the micromethod was less sensitive than the macromethod. We therefore prefer the macromethod, not only for studying the characteristics of the reaction but also

Address for reprints: E. Alessi, Clinic of Dermatology, University of Milan, Milan, Italy

Received for publication 8 July 1977

*Reagents from Fujizoki Pharmaceutical Co. Tokyo

Table 1 *Positive TPHA reactions in patients with primary and secondary syphilis (180 cases)*

Micromethod		Macromethod	
Dilution	%	Dilution	%
1/20	91.1		
1/40	88.3	1/80	91.6
1/80	74.4	1/160	83.8
1/160	67.2		

Table 2 *Positive TPHA reactions in patients with treated primary and secondary syphilis (299 cases)*

Micromethod		Macromethod	
Dilution	%	Dilution	%
1/20	94.9		
1/40	91.6	1/80	94.9
1/80	83.9	1/160	89.6
1/160	66.5		

for obtaining firm evidence in dubious cases; therefore we used the macromethod in our research. However, should it be necessary to resort to a micromethod—such as, for mass serological screening—we would use it at 1/20–1/40 serum dilutions, although these dilutions give a slightly higher number of false positive reactions and spontaneous haemagglutinations. As shown in Tables 1 and 2, the percentage of positive reactions obtained with the micromethod at the lowest dilutions is practically the same as that obtained with the macromethod at dilutions of 1/80–1/160.

Results and discussion

SENSITIVITY

We found that in the early stage of syphilis the sensitivity of the TPHA test was similar to that of the FTA–ABS test, the percentages of positive reactions found in the different groups of sera examined being practically identical.

Of 90 cases of untreated primary syphilis confirmed by darkfield examination (Table 3), we obtained 83.3% positive reactions with TPHA (1/80) and 81.1% positive reactions with FTA–ABS. In 90 cases of untreated secondary syphilis, all the sera were found to be positive with both reactions.

It also seemed worthwhile to study the relationship of time of the reactivity and compare it with other serological tests. Patients were divided into three groups according to date of infection (Table 4). It was observed that haemagglutinating antibodies appeared early; over 70% of the cases had positive results within 30 days of the infectious sexual intercourse (this percentage was the highest of all

the reactions examined). A second study of antibody titres carried out on a few positive sera (Table 5) revealed that the reaction was positive up to a dilution of 1 in 10 240 at the primary stage of the infection, and up to a dilution of 1 in 655 360 at the secondary stage.

The TPHA test remains positive for a long time, even in treated subjects. An investigation on 228 sera of patients with syphilis detected either at the primary or the secondary stage and examined one to 10 years after completion of treatment (Table 6) showed a higher percentage of positive reactions than with any other test, including FTA–ABS. We even found several positive reactions in patients who had acquired the infection 20 to 30 years before the serological tests.

Table 3 *Positive serological reactions (%) in primary and secondary syphilis*

	Primary syphilis (90 cases)	Secondary syphilis (90 cases)
RPCF	57.7	95.5
VDRL	62.2	100
TPI	28.8*	95.5†
FTA–ABS	81.1	100
TPHA 1/80	83.3	100

*Doubtful TPI test in another 16.6% of cases

†Doubtful TPI test in another 3.3% of cases

Table 4 *Positive serological reactions (%) in primary and secondary syphilis according to date of infection*

	Days from infection		
	<30 (35 cases)	30–40 (31 cases)	>40 (24 cases)
RPCF	31.7	32.2	95.8
VDRL	40	67.7	95.8
TPI	5.7*	38.7†	54.1‡
FTA–ABS	62.8	87	100
TPHA 1/80	71.4	80	100

*Doubtful TPI in another 8.5% of cases

†Doubtful TPI in another 16.1% of cases

‡Doubtful TPI in another 33.3% of cases

Table 5 *Positive TPHA reactions (%) at the different dilutions in positive sera of patients with primary and secondary syphilis*

Primary syphilis (32 cases)		Secondary syphilis (20 cases)	
1/80	100	1/320	100
1/160	78	1/5120	95
1/320	53	1/10240	75
1/640	44	1/20480	60
1/1280	25	1/40960	45
1/2560	12	1/81920	35
1/5120	9	1/163840	15
1/10240	3	1/655360	10

Table 6 Positive serological reactions in subjects with treated primary or secondary syphilis (tests performed 1 to 10 years after the end of treatment)

	Percentage of treated syphilis	
	Primary (113 cases)	Secondary (115 cases)
RPCF	0.8	4.3
VDRL	6.1	12.1
TPI	12.3*	36.5†
FTA-ABS	71.6	98.2
TPHA 1/80	88.4	100.0

*Doubtful TPI in another 18.5% of cases

†Doubtful TPI in another 20.0% of cases

SPECIFICITY

The treponemal haemagglutination reaction is attributed an extremely high specificity (Cox *et al.*, 1969; Tringali, 1970; Young *et al.*, 1974), comparable with that of the *Treponema pallidum* immobilisation (TPI) and FTA-ABS tests, although there are authors who question this (Garner *et al.*, 1973). Our experiments were on 559 patients (Table 7), who were in hospital because of various dermatoses—such as psoriasis, contact dermatitis, tumours, or tinea pedis. All patients were free from clinical symptoms of syphilis, the disease also being absent from their personal and family history. In a first group of 324 sera which gave negative Reiter protein complement fixation (RPCF), VDRL, and TPI reactions, nine (2.79%) were found to be TPHA positive. In another group of 235 sera, giving not only negative RPCF, VDRL, and TPI reactions but also a negative FTA-ABS reaction, one (0.4%) was TPHA positive. A comparison between the two groups clearly shows that, if a negative TPI result is regarded as sufficient to rule out the presence of syphilis, the percentage of false positive TPHA tests appears to be high; on the other hand, if a negative FTA-ABS test is also regarded as being necessary for syphilis to be ruled out, then that percentage is reduced to acceptable values.

We also examined 50 sera with positive VDRL and/or RPCF reactions and negative TPI and FTA-ABS results (Table 7). In this third group of sera, 4% of the patients were TPHA positive, as

Table 7 Positive TPHA reactions in clinically negative subjects (609 cases)

Group	%
First (324 cases) (RPCF, VDRL, and TPI negative subjects)	2.79
Second (235 cases)* (RPCF, VDRL, TPI, and FTA-ABS negative subjects)	0.4
Third (50 cases) (RPCF and/or VDRL positive but TPI and FTA-ABS negative subjects)	4.0

two VDRL positive sera were also found to be TPHA positive, probably owing to biological false positive results.

The problem of specificity is a difficult one as the patient's history is often unreliable, and the extremely high sensitivity of the reaction may cause positive reactions occasionally to be found many years after the infection when all other serological tests have become negative. Consequently, there are no reliable criteria for regarding a single positive TPHA result as non-specific.

Conclusion

In clinical practice the TPHA test is useful for serological screening, in combination with the VDRL reaction. In this case, because it is cheap, the micromethod at serum dilutions of 1/20–1/40 should be chosen and results evaluated as shown in Fig. 1.

It is also useful for diagnostic purposes, in patients with doubtful clinical history, symptoms, and serological results. In this case, the macromethod should be used as the higher dilution of sera reduces the incidence of inconclusive results caused by spontaneous haemagglutination in controls. It also reduces the incidence of false positive reactions. In this case, results will be evaluated in accordance with the scheme illustrated (Fig. 2).

VDRL	—	} No syphilis*
TPHA micromethod	—	
VDRL	+	} Syphilis? (continue the serological investigation according to scheme 2)
TPHA micromethod	+	
VDRL	—	} Syphilis? (continue the serological investigation according to scheme 2)
TPHA micromethod	+	
VDRL	+	} Syphilis? (continue the serological investigation according to scheme 2)
TPHA micromethod	—	

*The term 'no syphilis' is adopted for practical screening purposes only, since syphilis at a very early stage or long-since treated syphilis cannot be ruled out

Fig. 1 Scheme for the serological screening

FTA-ABS	—	} No syphilis
TPHA macromethod	—	
FTA-ABS	+	} Syphilis
TPHA macromethod	+	
FTA-ABS	+	} TPI
TPHA macromethod	—	
FTA-ABS	—	} TPI
TPHA macromethod	+	

— Biological false positive results or early syphilis or old syphilis
+ Possible technical error in one of the reactions

Fig. 2 Scheme for serological testing in subjects with suspected syphilis

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Improved management in the diagnosis of gonorrhoea in women

A. E. JEPHCOTT AND S. RASHID

From the Public Health Laboratory, Northern General Hospital and the Special Treatment Clinic, Royal Infirmary, Sheffield

SUMMARY In this paper an evaluation is made of the endeavours to improve diagnosis in women named as contacts of gonorrhoea. The problem was approached in three ways. (a) The number of sites sampled was increased. (b) The results of microscopical examination of smears made by clinical staff were regularly evaluated. (c) Cultural examinations by the supporting laboratory were dealt with more efficiently and speedily. It is concluded that the number of tests currently used to establish or exclude a diagnosis of gonorrhoea in women can safely be reduced to two, and that the methods currently in use allow a more cost-effective management of an increasing case load.

Introduction

Efficient diagnosis of gonorrhoea is essential to control the disease. Until recently it was usual to make three separate examinations of genital specimens before eliminating gonococcal infection in a female contact or in a suspected case. This can be a time-consuming and an unrewarding procedure. Recent advances in laboratory techniques have so improved diagnostic efficiency that it is doubtful if as many examinations are still necessary. Accordingly a review of the performance of the diagnostic service in Sheffield during the past eight years was made to establish the cost effectiveness and medical necessity of repeated examinations.

Patients and methods

The notes of all cases of gonorrhoea in women diagnosed during the first quarters of the years 1970-77 were reviewed to establish how the diagnoses had been reached and whether this had been at the first, second, or third examination. Cases in which the diagnosis was made at the third examination or later were reviewed in detail.

Routinely patients were examined on three occasions and samples were taken for microscopical examination and culture. The examinations were

made at the earliest opportunity, daily if possible. Initially, urethral and endocervical samples only were examined. Since early in 1974 rectal samples taken through a proctoscope have also been examined. From the same time, pharyngeal samples have been cultured selectively if examination of the first set of Gram-stained smears gave negative results or if there was a history of fellatio.

The methods of sampling for smear preparation, Gram staining, and microscopical examination were recommended by Morton (1977). All smears, both diagnostic and post-treatment, were read at the time of the patients' attendance. Until 1976 three follow-up sets of tests were routine. Thereafter two sets were used. All diagnostic smears were retained until the results of the corresponding culture specimens were available. In cases of disparate findings the smears were reassessed. This day-by-day appraisal of findings, together with periodic evaluation of smear and culture methods, was applied by medical and nursing staff during the eight-year study period.

The clinic and supporting laboratory are two miles apart. In the earlier years Stuart's transport medium was used to protect all specimens sent for culture. On reaching the laboratory they were plated on to a non-selective heated blood medium (McLeod *et al.*, 1934). A selective medium (Phillips *et al.*, 1972) was introduced late in 1970. Such media inhibit the growth of some gonococci (Reyn, 1973), so a selective medium and one that was non-selective were used in parallel for all samples submitted in transport medium after that date. Since

Address for reprints: Dr A. E. Jephcott, Public Health Laboratory, Myrtle Road, Bristol

Received for publication 31 October 1977

1974 Amies' transport medium has been used in preference to Stuart's.

In 1974 samples obtained at the first examination were inoculated directly on to a Transgrow transport-cum-culture medium. If second and third examinations were carried out Amies' transport medium was used (Jephcott *et al.*, 1974). Jembec transport-cum-culture medium replaced Transgrow for first examination samples in 1975 and has been in use ever since (Jephcott *et al.*, 1976).

Before routine use all new media were evaluated by using specimens from men with urethral Gram stains showing typical Gram-negative intracellular diplococci.

Suspicious colonies were submitted to Gram stain and oxidase testing and were finally identified by sugar fermentation (Flynn and Waitkins, 1972). Since 1974 an immunofluorescent (IF) method has been used routinely (Jephcott, 1977). The biochemical method is still used if the results from the IF method are unsatisfactory and for all rectal and pharyngeal isolates.

Findings

The Table shows the cases of gonorrhoea diagnosed in women during the first quarters of each of the eight years 1970-77. The number diagnosed by microscopical examination has remained relatively constant. This has resulted in a decrease in the proportion so diagnosed. There has been both an absolute and a relative increase in the numbers diagnosed by cultural methods. This is depicted graphically in Fig. 1 which also shows changes in methods. Figures 2-4 show the cases diagnosed at the first, second, and third tests by microscopy and culture. Figure 5 shows the proportion of cases diagnosed in the first two tests.

Throughout the entire study period seven cases (0.9%) were diagnosed at the third (or in one case the fourth) attempt. These cases are of special interest and the findings are germane to a detailed appraisal. They are therefore reported in detail.

PATIENTS REQUIRING THREE OR MORE TESTS

Patient 1

This patient was seen on 6 January 1970 as her consort had a urethral discharge. She was asymptomatic, her last sexual contact being the day before. Smears and cultures were negative. She attended again on 12 January reporting another exposure on 10 January. Tests were again negative but a third set of tests, taken on 13 January, revealed a positive cervical culture. Her consort was diagnosed on 12 January. She might have been infected on 10 January and not 5 January. It is therefore

Table Diagnosis of gonorrhoea in women by examining Gram-stained smears and by culture

Year	First test			Second test			Third test			Fourth test			Total cases diagnosed
	Gram	Culture	Total	Gram	Culture	Total	Gram	Culture	Total	Gram	Culture	Total	
1970	64 (90.1)	50 (70.4)	67 (94.4)	2 (2.8)	3 (4.2)	3 (4.2)	0	1 (1.4)	1 (1.4)	1 (1.4)	0	0	71
1971	62 (76.5)	58 (71.6)	70 (86.4)	9 (11.1)	5 (6.2)	14 (17.3)	0	0	0	0	0	0	81
1972	57 (67.8)	71 (84.5)	77 (91.7)	4 (4.8)	4 (4.8)	8 (9.6)	0	0	0	0	0	0	84
1973	59 (68.5)	62 (72.1)	73 (84.9)	8 (9.3)	10 (11.6)	18 (20.9)	0	0	0	0	0	0	86
1974	66 (64.7)	89 (87.2)	98 (96.1)	2 (2)	2 (2)	4 (3.9)	1 (1)	0	1 (1)	0	1 (1.2)	1 (1.2)	102
1975	64 (74.4)	73 (84.9)	78 (90.7)	5 (5.8)	4 (4.6)	9 (10.4)	3 (3.5)	2 (2.3)	5 (5.8)	3 (3.48)	0	0	86
1976	72 (64.9)	104 (93.7)	108 (97.3)	2 (1.8)	2 (1.8)	4 (3.6)	0	0	0	0	0	0	111
1977	71 (62.8)	109 (96.5)	111 (98.2)	1 (0.9)	1 (0.9)	2 (1.8)	1 (0.9)	1 (0.9)	2 (1.8)	1 (0.9)	0	0	113

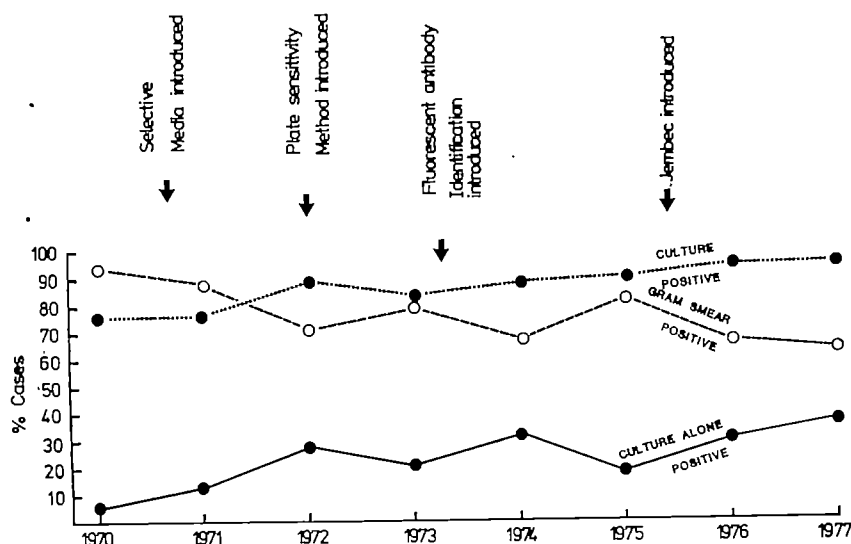


Fig. 1 Percentages of cases of gonorrhoea diagnosed by examination of smear or culture, January–March 1970–77

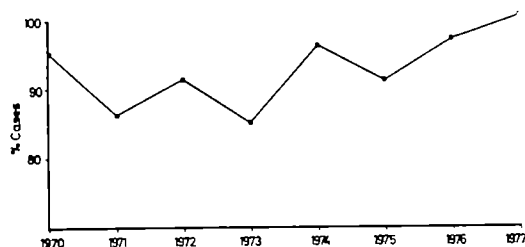


Fig. 2 Total number of women diagnosed in the first test

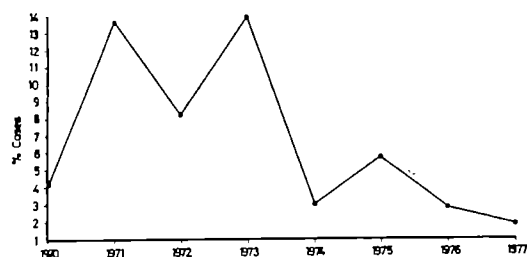


Fig. 3 Total number of women diagnosed in the second test

difficult to establish the number of examinations made after infection.

Patient 2

This patient was a prostitute. She first attended with a vaginal discharge on 14 March 1973, three days

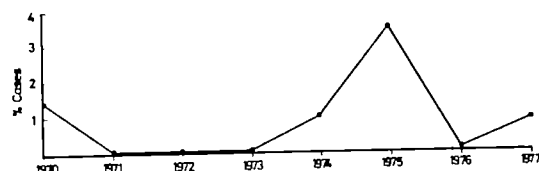


Fig. 4 Total number of women diagnosed in the third test

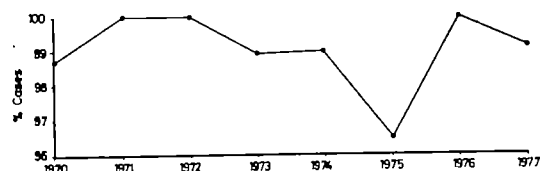


Fig. 5 Total number of women diagnosed with two tests

after her last sexual contact. She had had four different consorts during the previous month. The night before attending she had been given six antibiotic tablets (probably penicillin and analgesics) by her general practitioner. Three sets of tests on 14, 15, and 16 March gave negative results, but because symptoms persisted and treatment was inadequate a further set of tests was taken on 19 March. This time the cervical culture result was positive. Gonorrhoea was diagnosed in one of her consorts at his first attendance at the clinic on

20 March. Inadequate oral antibiotic treatment could have masked her infection while failing to eradicate it, or, she might not have been infected until between the 16 and 19 March—the case notes are unhelpful on this point.

Patient 3

This patient was seen initially on 18 January 1974 as the asymptomatic consort of a man with gonorrhoea who had been diagnosed and treated three weeks before. Examinations made on 18 and 22 January gave negative results but a rectal smear examination made on 24 January proved positive. All other smears and cultures gave negative results. Rectal smears are often difficult to read, but this smear was reassessed after the negative culture result became known.

Patient 4

This patient first attended on 27 December 1974 with symptoms. Tests taken on 27 and 30 December gave negative results but a third set, taken on 6 January 1975, yielded positive results to smears and cultures. Last sexual contact was said to be five months previously, although the symptoms were of recent onset. The history may be considered questionable in this instance.

Patient 5

This patient was one consort of a man who was diagnosed as having gonorrhoea and was treated on 20 January 1975. She attended on 21 January 1975, 10 days after her last sexual contact. Her history was confirmed by the consort. Tests taken on 21 and 22 January gave negative results but a rectal smear was found to be positive in the third set of tests taken on 23 January. All other smears and cultures taken that day gave negative results. The man had had one other consort but she did not attend the clinic. Again the difficulty of reading rectal smears is evident.

Patient 6

This patient attended on 23 January 1975, one week after contact with a man who was found to be suffering from non-specific urethritis. His urethral smear and culture showed no gonococci and he gave no history of recent treatment with antibiotics. The patient was examined on 22 and 24 January 1975 with negative results, but on 31 January cervical smears and cultures both proved positive. The patient admitted to no other consort during the previous six months.

Patient 7

This patient first attended on 12 February 1977 as

a contact of her husband who was diagnosed as having gonorrhoea and was treated on the same day. Her last marital contact was stated to be five days before and her husband confirmed this. Tests made on 12 and 14 February were negative, but both smears and cultures gave positive results at a third examination on 15 February. The patient denied any other contact. The husband had had a casual contact one week before attendance. It is possible that the first examinations were carried out during the wife's incubation period if the history is disregarded, but many other explanations are also possible in this instance.

Discussion

The number of examinations required to diagnose gonorrhoea in women has recently been discussed. Chipperfield and Catterall (1976) suggested that only two examinations were necessary. They did not use selective media or IF methods, however, and did not normally obtain rectal samples. Evans (1976) did use a selective medium but he did not use it in parallel with a non-selective one. He used IF identification but did not examine rectal specimens routinely. These two omissions are known to lead to missing between 3 and 5% of diagnoses (Reyn, 1973; Bhattacharyya and Jephcott, 1974). Thin *et al.* (1971) were among the first to use IF identification; they diagnosed 89% of their cases in two sets of tests.

There seems to be little doubt that the endeavours of supporting laboratories have effected improvements. This applies to isolation of gonococci and to the speed with which they can be identified by the IF method (Jephcott *et al.*, 1974). Analysis of the figures in this study shows that the first samples provided 98% of the diagnoses. This was increased to over 99% of the total when two tests were done.

A detailed review of cases diagnosed at a third examination (or later) suggests that there are reasons for late diagnosis. For example, the patient's history may be misleading (as for Patients 4 and 6), treatment with antibiotics may be insufficient to effect cure but may make difficulties (as in Patient 2), and there are suggestions that findings made on examination of Gram-stained rectal material are liable to be unconfirmed (Patients 3 and 5).

It is of interest to note that although the aim was to secure the attendance of all named contacts as early as possible and to repeat tests as near to daily as possible, there was no obvious failure owing to the patient being examined too soon, but Patient 7 suggests that this may theoretically be a possibility.

Conclusion and recommendation

It is concluded from this study that the methods currently in use in Sheffield warrant a reduction in the number of examinations for establishing or excluding the diagnosis of gonorrhoea in women. In the face of an ever-increasing case load we believe that the growing numbers can be efficiently managed by two sets of tests instead of the current three, provided that the following criteria are fulfilled:

- (a) The sites sampled routinely include the rectum, and the pharynx when indicated.
- (b) Gram-stained smear findings are continuously subjected to evaluation in terms of sampling, preparation, staining, and bacteriological findings.
- (c) While a selective transport-cum-culture medium offers a speedy diagnosis, its use should be confined to the first examination. Second samples should be submitted for culture on both selective and non-selective media.

In addition the methods outlined offer improved management for individual patients and more objective economical use of personnel and materials, and are likely to prove more cost-effective than their predecessors. Furthermore, increased efficiency and speed of diagnosis reduces the number of infectious 'patient-days' in the community and so makes a worthwhile contribution to disease control.

We wish to thank Dr R. S. Morton for advice and encouragement during the preparation of this manuscript.

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Studies on *Neisseria gonorrhoeae* cultured in liquid medium*

JULIA ANN EAVES

From Sheffield University Medical School

SUMMARY Experiments were carried out to determine the minimum amount of inoculum of gonococci that is needed to produce growth in ANM liquid medium. This was found to be less than had previously been thought. Minimum inocula were used to study penicillin sensitivity in liquid medium. This work showed that growth occurred at higher concentrations of penicillin with larger inocula. Evidence was also obtained which suggested that in some circumstances penicillin acts bacteriostatically rather than bacteriocidally. It was also shown that growth of gonococci can be inhibited in liquid medium by the presence of hyperimmune serum, the inhibition being more marked in the presence of fresh complement. This work is continuing in the hope that it may provide a basis for a much-needed serological test for gonorrhoea. This would be especially valuable in chronic asymptomatic infections.

Introduction

The gonococcus is a delicate organism which has a limited survival on solid medium. A new ANM liquid medium (Hafiz and McEntegart, 1976) which allows gonococci to survive longer was used to study four aspects of the growth of gonococci:

1. The smallest amount of inoculum needed to initiate growth.
2. The penicillin sensitivities in ANM liquid medium compared with those obtained using the plate method of Reyn *et al.* (1963).
3. The effects of adding penicillinase to cultures inhibited by penicillin.
4. The effect of specific antiserum on growth of gonococci in ANM liquid medium.

Materials and methods

ORGANISMS

Numbered stock strains that had been stored in liquid nitrogen and thawed were used. They were plated on Difco GC plates as required. Nine were originally obtained as fresh isolates from the Sheffield Special Clinic; the following international reference strains were also used: K₁ (Kellog), III, V, and VII.

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Received for publication 1 June 1977

MEDIA

The solid medium used was Difco GC medium. The ANM liquid medium was made up as described by Hafiz and McEntegart (1976) and distributed in volumes of 10 ml in universal containers. Corn or soluble starch was used and defined supplement (DS) could be added.

CULTURE

Inocula used in both plate and liquid cultures were 0.1 ml of serial dilutions of a dense suspension (optical density Brown's tube no. 5) of gonococci in phosphate buffered saline (PBS). The total number of organisms was confirmed by using Miles and Misra counts (Miles *et al.*, 1938). Cultures were incubated at 36°C and the CO₂ (10%) and humidity were automatically regulated.

PENICILLINASE

In studies of penicillin sensitivity in ANM medium an excess of penicillinase was added to all cultures in which penicillin had inhibited growth so as to determine if the penicillin was acting bacteriocidally or bacteriostatically.

PENICILLIN SENSITIVITY

1. The standard method described by Reyn *et al.* (1958) was used to determine the sensitivity of strains of gonococci to penicillin. International reference strains were used to produce a Kaber conversion table for correcting the values, as described by Reyn and Bentzon (1965).

2. Sensitivity in liquid medium—a modification of the plate method was used. Six penicillin dilutions in ANM liquid medium were made by adding stock solutions of penicillin in broth to ANM medium plus soluble starch and DS. Each dilution in the series was inoculated with gonococci at the determined minimum inoculum, two log dilutions above plus one below (making four in all), Table 2.

L-FORMS

Attempts to isolate L-forms were made by filtering ANM cultures and subculturing the deposit on Difco GC plates containing polyvinylpyrrolidone (Lawson and Douglas, 1973).

ANTISERUM

Hyperimmune rabbit serum with an antibody titre of 1:120 at a complement titre of 1:20 was produced by inoculating a rabbit with a suspension of gonococci (strain no. 55) for a period of four weeks. Two other rabbits were inoculated in a similar manner but they died before being bled.

This immune serum was used in an experiment designed to study its effect with and without added complement. Two sources of complement were used—Wellcome freeze-dried complement and fresh guinea-pig serum. Serum and both types of complement were millipore-filtered before use.

Dilutions of immune rabbit serum were doubled (from 1:20 to 1:320) in ANM liquid medium plus soluble starch plus DS. Controls and the following series were set up with the addition of 1 ml reconstituted one-fifth freeze-dried complement or 1 ml guinea-pig serum:

Series 1 Control—ANM+soluble starch (SS)+DS.

Series 2 Control—ANM+SS+DS+guinea-pig serum.

Series 3 Doubling dilutions immune serum+ANM+SS+DS.

Series 4 Doubling dilutions immune serum+freeze-dried complement in ANM+SS+DS.

Series 5 Doubling dilutions immune serum+guinea-pig serum (complement) in ANM+SS+DS.

All bottles were inoculated with 0.1 ml of a standard suspension of GC strain 55 in PBS (Brown's tube no. 5). Miles and Misra counts were done on this suspension. Bottles were incubated and subcultured on to plates at 24 and 48 hours.

Results

DETERMINATION OF THE MINIMUM AMOUNT OF INOCULUM OF GC IN ANM LIQUID MEDIUM
Four types of medium (see Table 1) were inoculated with serial doubling dilutions of standard suspen-

sions of gonococci: five strains were used. Miles and Misra counts were made to determine the sizes of inocula; the growth after 24 hours was determined in each bottle by subculturing on to plates. Results in Table 1 show that growth occurred with inocula as small as 10 organisms or less per 10 ml tube of ANM liquid medium.

ANTIBIOTIC SENSITIVITY VALUES

The antibiotic sensitivity values in liquid medium were compared with those from the routine plate method and the possible effects were investigated of penicillinase on cultures apparently inhibited by penicillin in liquid medium.

For each of 13 strains of gonococci the standard plate sensitivity was measured and the value in liquid medium was given by the penicillin concentration which inhibited the growth of a minimum inoculum of gonococci, the growth being assessed at 24 hours by subculturing on to plates. Penicillinase was added to bottles in which there was no growth after 48 hours; these were then subcultured at 72 hours.

Results for six strains are given in Table 2. They show that inoculum size may affect penicillin susceptibility, and in these cultures growth occurred after the addition of penicillinase.

To compare the two methods the values were arranged in rank order (see Table 3); this showed a correlation which appears to be statistically significant.

INHIBITION OF GROWTH OF GONOCOCCI IN LIQUID MEDIUM BY ANTISERUM

Growth of gonococci in ANM liquid medium was inhibited when hyperimmune serum was added. This was more marked when complement was also included. Two sets of results for strain no. 55 are shown in Table 4.

Discussion

MINIMUM INOCULUM

In 65% of cases growth occurred in 10 ml ANM liquid medium with fewer than 10 gonococci which is much less than had previously been thought (Hafiz and McEntegart, 1976).

Four different media were used and ANM plus soluble starch plus DS was chosen as the best medium for future work. Significantly, in the cases of two strains only one gonococcus was estimated to have been inoculated and this produced growth. The small amount of inoculum necessary has led to recent attempts to culture blood samples in liquid medium when looking for gonococcal septicaemia.

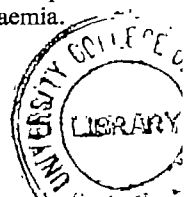


Table 1 *The growth of different inocula of gonococci in ANM liquid medium*

Strain no.	Inoculum and type of medium	Tube no. and dilution of suspension of gonococci in PBS						
		1 to 3 1 to 10 ⁻²	4 10 ⁻³	5 10 ⁻⁴	6 10 ⁻⁵	7 10 ⁻⁶	8 10 ⁻⁷	9 10 ⁻⁸
K2	Calculated no. of gonococci	Uncountable		710	245	10	1	—
	Growth in:							
	ANM+CS	+	+	+	+	+	+	—
	ANM+CS+DS	+	+	+	+	+	+	—
	ANM+SS	+	+	+	+	+	+	—
	ANM+SS+DS	+	+	+	+	+	+	—
97	Calculated no. of gonococci	Uncountable		1535	150	15	1	—
	Growth in:							
	ANM+CS	+	+	+	+	—	—	—
	ANM+CS+DS	+	+	+	+	+	+	—
	ANM+SS	+	+	+	+	+	—	—
	ANM+SS+DS	+	+	+	+	+	+	—
148	Calculated no. of gonococci	Uncountable		408	55	5	—	—
	Growth in:							
	ANM+CS	+	+	+	+	+	—	—
	ANM+CS+DS	+	+	+	+	—	—	—
	ANM+SS	+	+	—	—	—	—	—
	ANM+SS+DS	+	+	+	+	+	+	—
140	Calculated no. of gonococci	Uncountable		21	2	—	—	—
	Growth in:							
	ANM+CS	+	+	—	—	—	—	—
	ANM+CS+SS	+	+	+	+	—	—	—
	ANM+SS	+	+	—	—	—	—	—
	ANM+SS+DS	+	+	+	+	—	—	—
160	Calculated no. of gonococci	Uncountable		1140	80	8	1	—
	Growth in:							
	ANM+CS	+	+	+	+	—	+	—
	ANM+CS+DS	+	+	+	+	+	—	—
	ANM+SS	+	+	+	+	+	+	—
	ANM+SS+DS	+	+	+	+	+	—	—

CS= Corn starch
 SS= Soluble starch
 DS= Defined supplement
 ANM= ANM liquid medium
 + Growth on subculture at 24 hours
 — No growth on subculture at 24 hours

SENSITIVITY OF THE GONOCOCCUS TO PENICILLIN

1. There was a correlation between values of penicillin sensitivity obtained by the plate method and by using liquid medium but it is unlikely that liquid cultures will offer any advantage in routine work. This is because (a) contamination is likely to be more troublesome; (b) it is not possible to improve sensitivity; (c) the method is slower, taking 48 rather than 24 hours, and speed is critical in the treatment of gonorrhoea.

2. Inoculum size was shown to have an effect on susceptibility to penicillin. Growth occurred at higher concentrations if a larger inoculum was used.

3. In three strains out of 13, growth occurred in cultures only after penicillinase had been added. These were strains K₂, 151, and 170. In each case this happened at penicillin concentrations just above the previous limit for growth for each size of inoculum. This result was reproduced for strains 151 and 170.

Penicillin was here apparently behaving bacteriostatically yet it is normally described as bacteriocidal; this may have clinical relevance. It may be related to type as both strains K₂ and 151 are colony type 5 producing gonococci. An alternative suggestion is that growth after the addition of penicillinase was caused by reversion of L-forms which had survived in the presence of penicillin; however attempts to substantiate this by demonstrating L-forms using the electron microscope or by culturing filtered samples on plates with incorporated polyvinylpyrrolidone were unsuccessful (Lawson and Douglas, 1973).

INHIBITION OF GROWTH OF GONOCOCCI IN LIQUID MEDIUM BY INCORPORATING SPECIFIC ANTISERUM

Reproducible results were obtained for strain no. 55. It was shown that by incorporating specific rabbit antiserum growth of gonococci in liquid medium was inhibited, especially when small inocula were

Table 2 Results of the inhibition of growth of six strains of gonococci in ANM liquid medium by incorporating penicillin. Comparative penicillin sensitivities obtained by the plate method are given

Strain no.	Plate sensitivity IC ₅₀ (iu)	Corrected sensitivity IC ₅₀ (iu)	Innoculum size	Liquid culture Penicillin concentration in iu/ml					
				Growth on 24-hour subculture					
				1 1.0	2 0.25	3 0.06	4 0.015	5 0.004	6 Control
VII	—	0.0089	17 × 10 ⁶	—	—	—	+	+	+
			170	—	—	—	—	+	+
			17	—	—	—	—	+	+
			2	—	—	—	—	+	+
97	1.00	1.10	94 × 10 ⁴	+	+	+	+	+	+
			94.5	—	+	+	+	+	+
			9.5	—	+	+	+	+	+
			1.0	—	+	+	+	+	+
114	0.35	8.5	23.6 × 10 ⁶	—	+	+	+	+	+
			236	—	—	+	+	+	+
			23	—	—	—	+	+	+
			2.3	—	—	—	—	—	—
K2	0.022	0.0089	5 × 10 ⁵	—	—	—*	+	+	+
			5	—	—	—	—*	+	+
			0.5	—	—	—	—	—	—
151	Plates contaminated		38.5 × 10 ⁶	—	—	+	+	+	+
			3.8	—	—	+	—*	—*	+
			0.3	—	—	—	—*	+	+
170	Plates contaminated		10 × 10 ⁶	—	—*	+	+	+	+
			10	—	—*	+	+	+	+
			1	—	—	—	+	+	+
K2 (repeated)	—	0.0089	7 × 10 ⁶	—	—	—	—	+	+
			70	—	—	—	—	+	+
			7	—	—	—	—	+	+
			0.7	—	—	—	—	+	+
151 (repeated)	—	—	34 × 10 ⁶	—	—	+	+	+	+
			3	—	—	—*	+	—*	—
			0.3	—	—	—	—*	—*	—**
170 (repeated)	—	—	13 × 10 ⁶	—	+	+	+	+	+
			131.5	—	+	+	+	+	+
			13	—	—*	+	+	+	+
			1	—	—*	+	+	+	+

Inoculum size—gonococci per 10 ml calculated using Miles and Misra counts

*Growth only after penicillinase was added

**The anomalous growth in a control after penicillinase was added

Table 3 Comparison of sensitivities arranged in rank order

Sensitivities (iu)	Plate method	Liquid medium
8.5	114	112
—	97	97
—	V	V
—	167	114
—	112	167
—	*	151
—	*	170
—	III	III
—	VII	VII
—	K ₂	K ₂
0.0089	172	172

*Plate values for 151 and 170 not available

used. The inhibition was much more marked when fresh guinea-pig complement was also present but not if reconstituted freeze-dried complement was used.

Serology in gonorrhoea is of limited value because

reactions are often non-specific and there is a latent period between infection and a positive serological result. At present there appears to be no ideal method for the early detection of significant levels of antibody. This experiment shows that further study is needed into the possibility of using serum from patients to inhibit the growth of gonococci in liquid medium as an alternative to the methods currently available.

I am grateful to Difco for the studentship under which I was able to carry out this work. I am also grateful to Professor M. G. McEntegart, Dr S. Hafiz, and the staff of the Medical Microbiology Department, Sheffield University Medical School, Dr R. S. Morton of the Special Clinic, Royal Infirmary, Sheffield, and Dr A. E. Jephcott, Public Health Laboratory, Northern General Hospital, Sheffield, for providing the facilities for this work, and to Mrs H. Bland and Mrs H. Ashford for their help in typing the manuscript.

Table 4 *The inhibition of growth of gonococci in ANM liquid medium by incorporating hyperimmune serum and adding fresh complement*

Nature of liquid medium	Size of inoculum strain 55	Serum dilutions (titre 1/120)				
		1/20	1/40	1/80	1/160	1/320
Control ANM	Heavy (2 × 10 ⁶)	No serum	No serum	NT	NT	NT
	+	+	+	+	+	+
	Light (c 5)	No serum	No serum	NT	NT	NT
Serum in ANM	Heavy	+	+	+	+	NT
	+	+	+	+	+	NT
	Light	+	+	+	NT	NT
Serum and freeze-dried complement in ANM	Heavy	+	+	+	NT	NT
	+	+	+	+	NT	NT
	Light	—	—	—	—	—
Serum + fresh complement in ANM	Heavy	—	—	—	—	—
	+	—	—	—	—	—
	Light	—	—	—	—	—
Control ANM	Heavy (42 × 10 ⁴)	No serum	No serum	NT	NT	NT
	+	+	+	+	+	+
	Light (420)	No serum	No serum	NT	NT	NT
Control ANM + fresh complement	Heavy	No serum	No serum	NT	NT	NT
	+	+	+	+	+	+
	Light	No serum	No serum	NT	NT	NT
Serum in ANM	Heavy	+	+	+	+	+
	+	+	+	+	+	+
	Light	—	—	—	—	—
Serum + freeze-dried complement in ANM	Heavy	+	+	C	+	C
	+	+	+	+	+	+
	Light	—	—	—	—	—
Serum + fresh complement in ANM	Heavy	—	—	—	+	+
	+	—	—	—	+	+
	Light	—	—	—	—	C

Source of fresh complement was millipore filtered fresh guinea-pig serum

C = Contaminated

+ Growth on 24-hour subculture

— No growth on 24-hour culture

NT = Not tested

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The efficacy of cefuroxime for the treatment of acute gonorrhoea in men

J. D. PRICE AND J. L. FLUKER

From the West London Hospital, Charing Cross Hospital Group, London

SUMMARY This is a report on a clinical trial in which cefuroxime was used for treating 110 men with uncomplicated urethral gonorrhoea. Twenty-three men were given 1 g cefuroxime intramuscularly and 1 g probenecid orally, and 87 were treated with 1.5 g cefuroxime intramuscularly with 1 g probenecid orally. All 18 patients treated with 1 g cefuroxime and 1 g probenecid and seen at least once after treatment, were cured. Sixty-six (98.5%) of 67 patients treated with 1.5 g cefuroxime and 1 g probenecid and seen at least once after treatment, were cured. No side effects were reported. The high cure rates compared favourably with other antibiotics now in regular use and cefuroxime should be of great value especially for patients infected with β -lactamase producing gonococci.

Introduction

Since its introduction, penicillin has been the drug of choice for treating gonorrhoea as, until recently, most strains have been susceptible to low serum concentrations of penicillin. Since 1958 less sensitive strains of gonococci have been cultured and greater doses of penicillin have been necessary (Curtis and Wilkinson, 1958).

The Medical Research Council (1961) showed that 13.2% of gonococci had a minimum inhibitory concentration (MIC) of between 0.125 and 1.0 u/ml. Gray *et al.* (1970) at St Thomas's Hospital found that 35% of strains showed partial resistance (MIC $>$ 0.06 μ g/ml). At the Charing Cross Hospital, Beck (1973, personal communication) reported that 9% of penicillin-sensitive gonococci had an MIC of between 0.25 and 1.0 u/ml (0.15 to 0.6 μ g/ml) and 4% had an MIC above 1.0 u/ml.

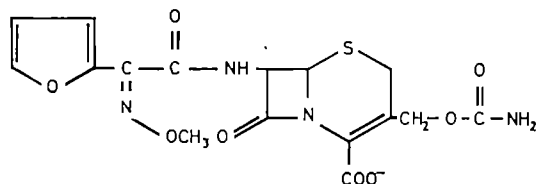
Gray *et al.* (1970), using 5 megaunits of benzylpenicillin in 8 ml lignocaine with 1 g probenecid orally in 200 patients treated in London, had one failure (0.5%) in the 185 who returned for follow-up. Olsen and Lomholt (1969) had a relapse rate of 1.0% in 832 cases similarly treated in Greenland. The Center for Disease Control (1974) in Atlanta, USA, recommended a single dose of either 4.8 megaunits of penicillin or 3.5 g ampicillin with 1 g probenecid for treating uncomplicated urethral gonorrhoea. Kvale *et al.* (1971) using 3.5 g ampicillin plus 1 g probenecid had a 4% failure rate in

the Philippines. Price *et al.* (1977) giving 1.4 g talampicillin had a 1.6% failure rate in London.

With the appearance of β -lactamase producing strains of gonococci, the effectiveness of the β -lactam antibiotics is being questioned (Ashford *et al.*, 1976). Cefuroxime, a new β -lactamase stable cephalosporin, has been developed which, from *in vitro* sensitivity tests in Liverpool (Percival *et al.*, 1976) and at St Thomas's Hospital, London (Phillips *et al.*, 1976), is effective against β -lactamase producing gonococci.

Cefuroxime is a semisynthetic analogue of cephalosporin C and, as shown in the formula, has a methoxyimino group and a furyl group on the side-chain attached to the nucleus at position 7 (Figure). This stabilises the β -lactam ring against the bacterial β -lactamases. Cefuroxime has a broad spectrum of activity and is resistant to β -lactamases produced by Gram-negative organisms (O'Callaghan *et al.*, 1976). Cefuroxime is excreted unchanged in the urine.

Cefuroxime is not absorbed orally and has to be given intramuscularly or intravenously. The serum concentrations are dose related and a peak concentration of 40 μ g/ml is reached half to one hour after



Cefuroxime

Figure Cefuroxime structural formula

Address for reprints: Dr J. D. Price, Glaxo Holdings Limited, Medical Department, Graham Street, London N1 8JZ

Received for publication 4 July 1977

giving 1 g intramuscularly. Levels can still be estimated after eight hours (Foord, 1976) and 33% is bound to serum protein.

We have assessed the efficacy and tolerance of two different single dose levels of cefuroxime with oral probenecid for the treatment of acute uncomplicated urethral gonorrhoea in men.

Method

Men suffering from urethral gonorrhoea provisionally diagnosed by the appearance of typical diplococci in a Gram-stained smear were included in the study, provided that they were not known to be allergic to cephalosporins or to have had a previous anaphylactic reaction to penicillin. Cultures on modified Martin Thayer medium (Riddell and Buck, 1970) were incubated for bacteriological identification and MIC determination. The patients were then given cefuroxime by intramuscular injection, the 1 g dose being dissolved in 4 ml of water for injection, the 1.5 g dose being given as 750 mg dissolved in 3.0 ml into each buttock. All patients were instructed to refrain from alcohol for the first week after the injection and from sexual intercourse until completion of the review. Patients were asked to return after 4, 7, and 14 days for follow-up assessment, or at any time if the symptoms returned. At each follow-up visit a history was taken and the patient examined. Samples of deposit from centrifuged urine and deep intraurethral exudates were stained and examined in the clinic.

Results

One hundred and ten men who were attending the clinic with acute uncomplicated gonorrhoea were randomly included in the study after positive identification of Gram-negative diplococci in the urethral smear. The first 23 patients received 1 g cefuroxime, and the next 87 patients received 1.5 g cefuroxime. All received 1 g of probenecid at the same time as their injection.

Eighty-four patients were between 18 and 30 years of age, 79% were single, only 16% were married, and 5% were either divorced or separated. Sixty-eight per cent of the patients were Caucasian, 29% Negroid, and 3% Asian.

Table 1 MIC of isolated organisms

Cefuroxime $\mu\text{g/ml}$	Penicillin G ($\mu\text{g/ml}$)					Total
	0.0004-0.005	0.006-0.01	0.011-0.05	0.06-0.1	0.11-0.5	
0.0004-0.005	11	1	1	—	—	13
0.006-0.01	7	6	—	—	—	13
0.011-0.05	—	3	3	2	1	9
0.06-0.1	—	—	—	—	1	1
0.11-0.5	—	—	—	—	1	1
Total	18	10	4	2	3	37

Two patients had already received antibiotics. One had received 3 g ampicillin and 1 g probenecid orally which had failed to cure his gonorrhoea and the other patient had been given different antibiotics but was repeatedly reinfected by the same contact. Cefuroxime was followed by cure in both these cases.

BACTERIOLOGICAL RESPONSE

Cultures from 37 of the 110 cases were sent to the Microbiology Department, Glaxo Research for MIC determination. Their sensitivity to penicillin G showed that three organisms had an MIC of between 0.11 and 0.5 $\mu\text{g/ml}$ (Table 1).

Of the 23 patients treated with 1 g cefuroxime and 1 g probenecid, 18 returned for at least one follow-up and in all 18 cases *Neisseria gonorrhoeae* was successfully eradicated.

In the second group of 87 patients given 1.5 g cefuroxime and 1 g probenecid, 67 patients returned for follow-up, 60 were successfully treated, two were confirmed reinfections, and in one, treatment failed. Forty patients returned for a second follow-up and 10 patients returned for a third time. The 23 unassessable patients had no microbiological sample taken after the initial visit. In all, treatment was clinically successful.

In these 110 cases treated, 80 patients were assessable. The overall bacteriological response was only one failure—98.5% (Table 2).

CLINICAL RESPONSE

The clinical response was assessed as follows:

Table 2 Bacteriological response

Result of treatment	No. of patients treated with		Total
	1 g cefuroxime and 1 g probenecid	1.5 g cefuroxime and 1 g probenecid	
Successful	17	60	77
Successful with reinfection	1	1	2
Failed	—	1	1
Reinfection	—	2	2
Unassessable*	5	23	28
Total assessable	18	62	80

*Neither smear nor culture taken after first visit

Table 3 Clinical response

Result of treatment	No. of patients treated with		Total
	1 g cefuroxime and 1 g probenecid	1.5 g cefuroxime and 1 g probenecid	
Cured	13	58	71
Cured with reinfection	1	—	1
Improved (PGU)	3 (17.6%)	2 (3%)	5
Unassessable	6	27	33
Total assessable	17	60	77

(1) Cured with complete remission of all signs and symptoms. (2) Cured with subsequent reinfection. (3) Symptoms improved but signs of post-gonococcal urethritis (PGU) present. (4) Unassessable if initially asymptomatic, or if symptoms present within 14 days of treatment and the patient given other treatment or was not followed up.

Eighteen of the patients treated with 1 g cefuroxime and 1 g probenecid returned for a first follow-up. If patients did not return for a follow-up 14 days after treatment, clinical assessment was made according to the information from the last visit. This is shown in Table 3.

In the second group of patients (that is, those given 1.5 g cefuroxime and 1 g probenecid) 67 patients returned for at least one follow-up. Fifty-eight patients had relief of all signs and symptoms.

Five patients had PGU. In those treated with 1 g cefuroxime and 1 g probenecid, PGU occurred in three of 17 patients (17.6%). In those treated with 1.5 g cefuroxime and 1 g probenecid, PGU occurred in two of 60 patients (3%). In Table 3 the clinical response and the incidence of PGU is shown.

SIDE EFFECTS

No side effects were reported, even in the four patients who had a history of antibiotic allergy. Mild transient pain was noted in all cases on injection.

Discussion

When drugs other than penicillin are used to treat gonorrhoea, it is essential that comparable success rates be obtained. Patients with a known hypersensitivity to penicillin, or those in whom syphilis may be present, single dose treatment with co-trimoxazole and spectinomycin is used.

Carroll and Nicol (1970) in a trial of 111 cases using co-trimoxazole had a failure rate of five (4.5%). Reyn *et al.* (1973) had one failure in 109 cases. Kanamycin 2 g by injection as reported by Wilkinson *et al.* (1967), Hooton and Nicol (1967), and Fluker and Hewitt (1970) gave failure rates of about 3 to 5%. Siboulet and Durel (1961) in Paris

using spiramycin 2.5 g had 20 failures (3%) out of 594 patients.

Compared with these results, cefuroxime at 1.5 g has a very good success rate.

We wish to thank the members of the medical and nursing staff in the Luke Clinic, West London Hospital for their help in obtaining and treating the patients, Dr A. Gerkin and Mr D. Platt at the West London Hospital laboratories, Miss M. Marshall and Miss M. Kennedy for their bacteriological help, and Miss M. Rees for her evaluation of the data and Miss J. Bush for secretarial assistance.

We should also like to thank Miss B. Mullinger and Glaxo Research Limited for supplies of cefuroxime.

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Absence of detectable local antibody in genitourinary tract secretions of male contacts of women infected with *Trichomonas vaginalis*

J. P. ACKERS, R. D. CATTERALL, W. H. R. LUMSDEN, AND A. McMILLAN*

From the Department of Medical Protozoology, London School of Hygiene and Tropical Medicine; James Pringle House, Middlesex Hospital, London; and the Royal Infirmary, Edinburgh

SUMMARY Samples of semen and urine were obtained from 37 male contacts of women with proved *Trichomonas vaginalis* infection; on culture, eight (22%) of the men were shown to harbour the parasite. However, significant amounts of antitrichomonal antibody were found in only two of these samples, and the amounts present were very small. A further 10 samples were tested but none was found to contain antibody. The asymptomatic nature and low parasite numbers commonly described in infections in men is thus unlikely to be due to a vigorous local immune response.

Introduction

We have already demonstrated the presence of specific antitrichomonal antibody in the vaginal secretions of about 75% of a group of infected women as well as in 40% of a small group of controls (Ackers *et al.*, 1975). Previously, Chipperfield and Evans (1972) showed an increase in the number of immunoglobulin-bearing plasma cells in cervical biopsies, not only from women with gonorrhoea, trichomoniasis, and candidosis, but also in biopsies from women who had been exposed to *Neisseria gonorrhoeae* but who had not apparently become infected. Although it is believed that most cases of trichomoniasis are sexually transmitted (Catterall and Nicol, 1960), the parasite is usually difficult to demonstrate in the male. We therefore decided to examine various secretions from men who had been in recent contact with women with proved trichomoniasis to see if any evidence of local antibody production could be found.

Materials and methods

SAMPLES

Three types of specimen were collected—semen and urine from patients at James Pringle House, Middle-

sex Hospital, London, and semen and urethral secretions from patients attending the Department of Venereology, Royal Infirmary, Edinburgh.

Samples collected in London

Women with proved trichomoniasis were asked to invite their male contacts to attend. In all cases trichomoniasis was diagnosed by wet-film examination and confirmed by culture. Those men who did attend were given a routine examination and a urine sample was collected. They were then asked if they would provide a specimen of semen, either at the clinic or in their own homes. Those who chose the latter were asked to bring the specimen to the clinic with the minimum possible delay. Samples of semen and midstream urine were collected from the clinic twice a day, centrifuged (1000 *g* for 10 min) and the supernatants stored at -20°C. Sediments were examined by wet film and culture for the presence of *T. vaginalis*.

Samples collected in Edinburgh

The procedure was similar to that employed in London, all male contacts who attended being referred to one of the authors (AM). To collect urethral secretions, 2 ml of sterile phosphate-buffered saline were instilled into the distal urethra by means of a soft polyethylene tube. After allowing 60 seconds for equilibration with urethral secretions, the saline was collected in a sterile bijou bottle. If patients agreed, semen samples were collected at the clinic. Examination, centrifugation, and culture were

Address for reprints: Dr J. P. Ackers, Department of Medical Protozoology, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT

*Present address: Black Street Clinic, The Royal Infirmary, Glasgow

Received for publication 23 November 1977

carried out at the clinic; samples were sent, at -80°C , to London for antibody assay.

Procedures and medium used for culture were as previously described (Ackers *et al.*, 1975) except that, in view of the paucity of organisms expected, cultures were kept at 37°C for 14 days before being discarded.

ANTIBODY ASSAY

Preparation of ^{125}I -labelled antihuman IgA and its use in the antibody assay were as previously described (Ackers *et al.*, 1975), modified slightly to eliminate the high values previously discussed for counts bound in the absence of antibody.

In the previous assay, each woman's secretion was assayed against her own isolate of *T. vaginalis*; however, this was not possible in this case since most of the men were culturally negative. To overcome this problem, and to make the assay more reproducible, a mixed antigen was prepared. Twelve strains, obtained from both London and Edinburgh, were grown in bulk and the harvested organisms washed and pooled. The organisms were then suspended in a mixture of inactivated calf serum (Wellcome) (10% v/v) and dimethylsulphoxide (Analar) (10% v/v) in phosphate-buffered isotonic saline, pH 7.2 (PBS) at a count of 5×10^6 organisms/ml and divided into 1 ml aliquots. These were then slowly cooled at -80°C and stored until required. After thawing, up to half the organisms were still visibly motile; they were washed three times with PBS, adjusted to 5×10^6 organisms/ml and used in the assay as previously described.

As before, antibody in the samples is expressed as counts per minute (cpm) bound. The significance of differences observed was assessed by calculating *P* values (Swinscow, 1976).

Results

In the London study, 36 men who were contacts of 260 women with microscopically proved *T. vaginalis* infection attended. Urine specimens alone were obtained from six and semen and urine from the other 30.

Specimens of semen and urine were obtained from three further men who had attended unasked: one had been in contact with a woman who had trichomoniasis, while the other two had frank urethral discharge and were shown by wet-film examination to be infected with *T. vaginalis*. Results of tests on these two men with symptomatic infection are presented separately at the end of this section.

Microscopical examination of sediments of semen and urine from the 37 contacts was uniformly

negative, but *T. vaginalis* was cultured from eight (22%) of the men—from semen alone in two cases, from urine alone in two, and from both semen and urine in the four others.

Of the 37 men in contact with infected women, three (including two shown to be infected with *T. vaginalis*) were already attending James Pringle House for the treatment of other complaints. Of the remaining 34 men, three (none apparently infected) had mild symptoms (urethral discharge, discharge and dysuria, and frequency respectively) and one, infected with *T. vaginalis*, was initially diagnosed as suffering from non-specific urethritis. The remainder (including five with proved infections) were completely asymptomatic.

An attempt was made to assess the degree of infection of the female partners of the men exposed to infection by counting the number of organisms in 10×400 fields of the initial wet film. Of the 260 women scored, 242 had a mean of 2.53 organisms/field (SD 2.433), while 18 (6.9%) had very significantly ($0.001 < P < 0.003$) higher scores (more than 10 organisms/field). The scores of 34 of the women whose contacts came forward were available: they had a mean of 1.85 (SD 2.1) organisms/field, not significantly different from the rest of the women. Two (5.8%) of these 34 women had high (> 10 organisms/field) scores, but neither had apparently infected her partner.

The Edinburgh clinic yielded 10 semen samples from known male contacts of infected women; none contained *T. vaginalis* as judged by culture or wet-film examination. A further nine samples, of semen and urethral secretions, were collected in Edinburgh from men who had no known contact with the parasite; *T. vaginalis* was not seen or cultivated from any of them.

Antitrichomonal antibody assays were performed on 23 semen and 14 urine samples from London contacts, and on all the Edinburgh samples. Results (mean cpm bound, corrected for radioactive decay) were: London, semen samples 216 (SD 161), urine samples 100 (SD 208); Edinburgh contacts 247 (SD 189); Edinburgh not in contact 270 (SD 297) (results from semen and urethral secretions were very similar, so have been combined). These mean values are not statistically different from one another, and all are far lower than the results found with vaginal secretions from infected women (Ackers *et al.*, 1975). Among all the samples tested, only two semen (both from London) contained significant ($P < 0.001$) amounts of antibody (1521 and 1773 cpm): *T. vaginalis* could not be isolated from either. The antibody content of those samples from which *T. vaginalis* was grown was not significantly higher or lower than that of the remaining ones.

In the absence of sufficient quantity of any sample to act as a reference preparation, a 1:5 dilution of a standard serum (antitrichomonal agglutinin titre 1:2 to 1:8) was included in each assay of the London samples. The mean counts bound for this standard from all the assays performed was 543 cpm (SD 200), and all assays in which the standard result was less than 250 or greater than 800 were discarded. An attempt was also made to increase the reproducibility of the assay by multiplying the measured counts so as to bring the counts bound by the standard serum to 500 in all cases. These 'corrected' results, however, were not greatly different from the uncorrected ones—from London, semen 158 cpm (SD 149), urine samples 112 cpm (SD 216). Using 'corrected' figures, three semen samples now appeared with significant ($P < 0.001$) quantities of antibody—the original two (1845 and 1008 corrected cpm) and a third with 1138 corrected cpm.

Semen samples from the two men who presented with symptomatic trichomonal urethritis were also assayed: their content of antitrichomonal antibody (933 and 860 uncorrected cpm; 530 and 609 corrected cpm) was just significant ($0.01 < P < 0.05$).

Discussion

The first part of this study, although only for a small number of men, confirms previous reports that *T. vaginalis* is difficult to isolate from men with known exposure to the parasite, and that even men with culturally-proved infection are frequently asymptomatic. It seems that the few women who had large numbers of organisms in their vaginal secretions were not particularly likely to produce a frank infection in their partners.

Having previously shown (Ackers *et al.*, 1975) that a significant number of infected women produced local antibody directed against this parasite, we wondered if the frequent absence of detectable infection in men was due to a similar local immune response. This, however, does not seem to be the case, for only two of our 44 men in contact had over 600 cpm of bound antibody, and the results (1521 and 1773 cpm) were far below the average figure (17 900 cpm) previously found in antibody-producing women (Ackers *et al.*, 1975). In view of the improvements made in the assay procedure, it seems unlikely that local antibody is produced by a significant number of male contacts, and, although the results from the two men with symptomatic infections were slightly above the average, they do not really suggest a vigorous immune response in progress.

An attempt was made to improve the reproducibility of the assay by relating all results to a common standard; results corrected by this procedure

support the conclusions drawn from the uncorrected ones.

A large amount of work has been done on the immune response of the female genital tract to various antigens, largely stimulated by an interest in the immunological aspects of reproduction and fertility control (for a review, see Cinidar and de Weck, 1976). In contrast, the male genitourinary tract has received little attention. Rümke (1974) showed that most of the immunoglobulin in semen came from the prostate, and that, while the IgG appeared to be derived from serum, there was evidence for a small amount of local IgA synthesis. Kearns *et al.* (1973) showed the presence of antigenococcal antibody in the urethral exudate of men with uncomplicated gonorrhoea. Tapchaisri and Sirisinha (1976) confirmed this for some of the men they examined, but they also found that the antibody levels were much higher in women. Similarly, in a study of local antibody production in children with urinary-tract infections, Jodal *et al.* (1974) found that very little local IgA antibody against *E. coli* was produced by the small number of boys in the sample, although they emphasised that they were the youngest children in the group. Finally, it should be noted that, in contrast to cows, bulls infected with *Trichomonas foetus* produce very little, if any, antibody (Robertson, 1963), although local infection with *Brucella abortus* does lead to a local response (Christensen, 1948): this infection produces considerable inflammation and tissue destruction. The lack, in men, of a vigorous immune response to such a scanty and non-invasive parasite as *T. vaginalis* is thus perhaps not surprising, especially as it is not clear how long the organisms remain in the male urethra after infection. It is quite possible that the repeated passage of urine mechanically removes the parasite within a few days. Certainly we were able to isolate the organism from urine as well as from semen, and an investigation of the length of time men remain infected could perhaps be combined with a study of the possible value of a routine urine culture in diagnosis.

This work was supported by a Project Grant from the Medical Research Council to J. P. Ackers. We are grateful to Sister Coladangelo and her staff for counting the trichomonads in wet films, to the doctors from both centres for their co-operation, and to Miss Shirley Smith and Mrs Alison Matthews for technical assistance.

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Specific immunity to human papilloma virus (HPV) in patients with genital warts

J. VIAC, M. J. STAQUET, M. MIGUET, M. CHABANON, AND J. THIVOLET

From Fra Inserm No. 11 Recherche Dermatologique et Immunologie Clinique Dermatologique, Hôpital Edouard Herriot, France

SUMMARY A study of human papilloma virus (HPV) specific cellular and humoral immunity in 30 patients with genital warts is reported. By *in vivo* testing with purified, inactivated plantar wart virus, a cell-mediated immunity to HPV was determined in 60% of patients. Circulating antibodies, evaluated by immunofluorescence testing, were rare, but these increased after an intradermal test had been carried out, especially in patients with a positive skin test, suggesting a booster effect. No significant difference was found between this group of patients and those having skin warts. Our results showed a specific immune response to HPV in most patients, confirming the role of the viral agent in the induction of genital warts.

Introduction

The viral aetiology of genital warts has now been well established by the presence of virus particles with typical human wart virus morphology (Dunn and Ogilvie, 1968; Oriel and Almeida, 1970) in these lesions. However, there is no evidence as yet of antigenic identity between the viruses found in different clinical types of wart in man (Almeida *et al.*, 1969) and very few studies have been undertaken on specific immunity to human papilloma virus (HPV) in patients with genital warts. Most studies carried out on humoral immunity have been on patients suffering from skin warts (Ogilvie, 1970; Cubie, 1972; Pyrhönen and Penttinen, 1972) and specific cellular immunity studies have been performed using only *in vitro* tests (Morison, 1974; Ivanyi and Morison, 1976; Lee and Eiseinger, 1976). This study was therefore aimed at determining the levels of specific humoral and cellular immunity in patients with genital warts before and after an *in vivo* test (intradermal test), using purified, inactivated HPV prepared from plantar warts, and comparing them with results obtained in a normal infected population (Thivolet *et al.*, 1977).

Material and methods

PATIENTS

The group studied comprised 25 Caucasians and 5 Arabs (19 males, 11 females). They had had genital warts for an average period of five months (minimum 1 month, maximum 15 months). None had a history of skin warts. Their average age was 26 years (minimum 16 years, maximum 55 years).

Forty adults and 23 children with various dermatoses but no clinical evidence or history of cutaneous viral warts were used as controls.

VIRUS PURIFICATION

HPV was obtained from plantar warts, acquired after surgical excision, immediately frozen in liquid nitrogen and stored at -25°C . This HPV was purified according to the procedure described by Pass and Marcus (1973) and inactivated with 0.4% formalin, dialysed against phosphate buffer solution (PBS), and diluted to approximately 10^{10} particles per 0.1 ml (5 μg proteins). Controls were set up according to a method previously described (Thivolet *et al.*, 1977) (Fig. 1).

INTRADERMAL TEST (IDT)

The patients were injected intradermally with 0.1 ml HPV suspension containing 5 μg virus protein. They were examined after 24, 48, and 72 hours. Erythema

Address for reprints: J. Viac, Fra Inserm No. 11 Clinique Dermatologique—Hôpital Edouard Herriot, 69374 Lyon Cédex 2, France

Received for publication 5 November 1977

and induration were considered as indications of a positive test after a minimum of 24 hours.

HUMORAL IMMUNITY TEST

Sera were taken from patients just before IDT and eight days after. Indirect immunofluorescence (IF) tests were then performed as described by Genner (1971) (Fig. 2). In these tests sheep antihuman IgG and antihuman IgM conjugates (Behring-Hoechst) were used after absorption and dilution. Frozen sections of plantar warts, which showed specific nuclear fluorescence with a rabbit HPV antiserum (Viac *et al.*, 1977b) were used to detect antibodies in the patients' sera. The specificity of the IF test was shown by the absence of staining in a pre-immune rabbit serum and in human sera derived from children under the age of five years, who did not have warts. Sections of normal adult skin, exposed to the positive sera, also failed to yield fluorescence. All sera were tested at a dilution of 1:10 and slides were viewed with a Leitz fluorescence microscope (epi-illumination Orthoplan).

STATISTICAL ANALYSIS

Comparison between the observed percentages was based on the χ^2 test and the degree of significance was derived from the χ^2 table of Fisher and Yates.

Results

CELLULAR IMMUNITY TEST

The percentage of positive IDT in patients with genital warts is shown in Table 1. This percentage (60%), similar to that obtained from patients with plantar warts (64.3%), is higher than that seen in patients with hand (42.9%) or flat warts (41.8%). However, there is no statistical difference between these different values. The χ^2 values between genital warts and hand or flat warts were respectively 2.29 and 1.74. The percentage of positive IDT among all patients with different types of warts (52.5%) is

Table 1 *IDT in patients with genital warts: comparison with patients with other types of warts and with controls*

Type of case	No. of cases	IDT positive	Positive (%)
Warts			
Genital	30	18	60.0
Plantar	30	19	64.3
Hand	49	21	42.9
Flat (face or hand)	12	5	41.8
Controls			
Children aged 1-5 years	23	0	0.0
Others	40	4	10.0
Total	63	4	6.3

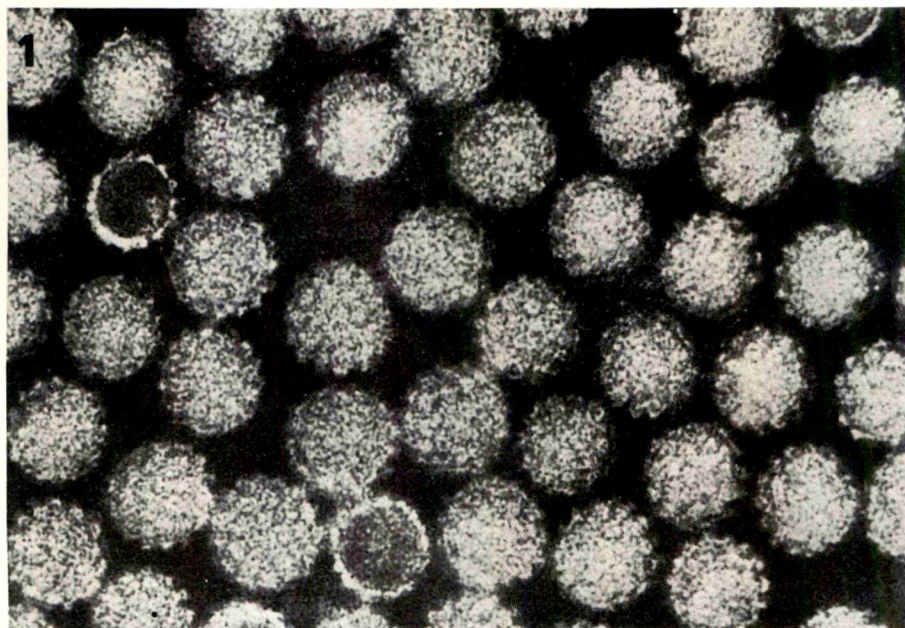


Fig. 1 *Electron micrograph of HPV particles purified according to the procedure described by Pass and Marcus (1973) and observed after negative staining with 2% phosphotungstic acid buffered to pH 7.0 $\times 250\,000$*

statistically higher than that of the control group (6.3%) (χ^2 value = 36.2). The control group comprised 40 adults, of whom four showed positive IDT, and 23 children aged 1-5, all of whom had negative results.

HUMORAL IMMUNITY TEST

The incidence of circulating antibodies was low before IDT (27.6%) but increased significantly after the skin test, to a value of 75% (χ^2 value = 11; $P=0.0001$). Most of the antibodies detected were of the IgM class (Table 2). Patients with a positive result to the skin test had a better seroconversion (86.6%) than patients with a negative result (55.5%); they also had a spontaneous incidence of antibodies greater than the negative group (Table 3).

Table 2 Incidence of circulating antibodies (IF test) in patients with genital warts

Time	No. of sera	Positive sera			P level
		IgG	Total (IgM, IgG)	Positive (%)	
Before IDT	29	2	8	27.6	0.001
After IDT	24	7	18	75.0	

Discussion

A few studies have been carried out on specific immunity in patients with genital warts. Cell

Table 3 Correlation between IDT and circulating antibodies

IDT	Before skin test			After skin test		
	No. of sera		Positive (%)	No. of sera		Positive (%)
	Positive	Total		Positive	Total	
Positive	7	19	36.9	13	15	86.6
Negative	1	10	10.0	5	9	55.5

mediated immunity to HPV has been studied by *in vitro* tests (Morison, 1974; Ivanyi and Morison, 1976), but there is much controversy regarding the significance of these tests (Lee and Eiseinger, 1976) and, in general, patients with anogenital warts were excluded from these studies (Morison, 1974).

Other authors (Maderna, 1934; Biberstein, 1944) tested skin hypersensitivity in patients with genital warts, but they used a saline extract of wart tissue, rather than purified HPV.

In this study, cellular immunity to wart virus has been tested directly by an IDT, using purified inactivated HPV. As already shown, the skin test we used seemed to be specific and sensitive in preliminary sera (Thivolet *et al.*, 1977). The incidence of positive IDT in patients with genital warts was as high as in patients with plantar warts and showed the existence of a cell-mediated immunity to HPV within these patients.

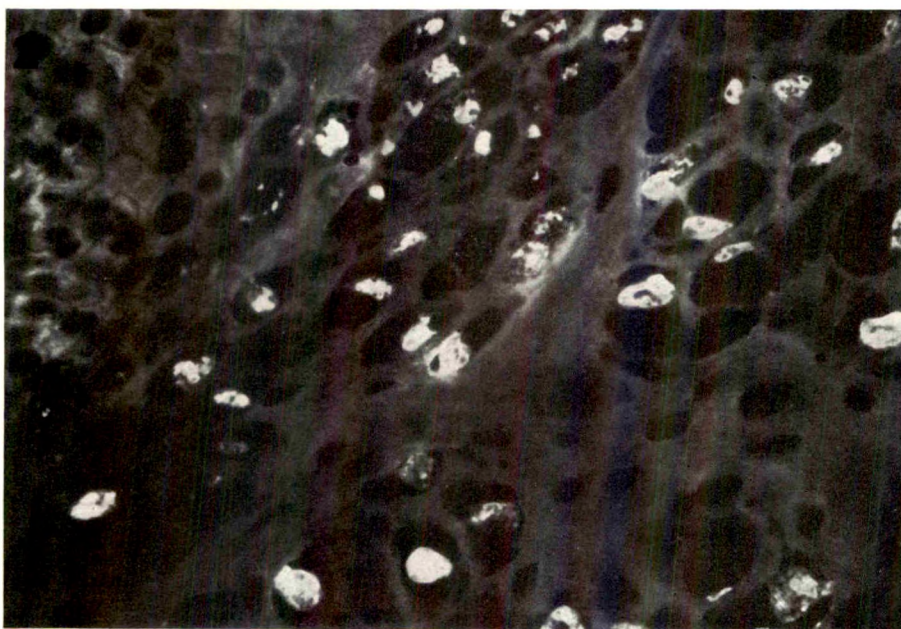


Fig. 2 Cryostat section of plantar wart. $\times 40$. Nuclei containing viral antigen. Stained with sheep antihuman IgG conjugate

Serological studies have demonstrated the existence of wart virus specific antibodies in the sera of patients with genital warts (Ogilvie, 1970). However, Almeida *et al.* (1969) found a one-way cross-reaction in such sera, which reacted with virus only from genital warts and not with that from skin warts, while sera from skin warts reacted with both types of virus, but more strongly with the homologous virus. In our study, a few sera (27.6%) had antibodies detectable by IF testing on plantar warts; this small percentage can be explained by the difference in antigenic affinity between plantar and genital wart viruses, or by the lower antigenic stimulus received in such patients, due to the lower number of virus particles present in genital warts (Ogilvie, 1970). The seroconversion observed after the skin test (27.6–75%), especially in patients with a positive skin test (36.9–86.6%) was in favour of wart virus specific antibodies present at low levels in many cases.

Thus, genital warts induced a specific immune response to HPV: a cell mediated immunity which seemed to play a major role in the cure of skin warts (Viac *et al.*, 1977a) and, less frequently, wart virus specific antibodies, which increased significantly after the skin test, thus acting as a booster effect.

Our results were similar to those obtained with patients bearing skin warts (Viac *et al.*, 1977b) and confirmed the existence of a host immune response directed toward the genital wart inducing viral agent (HPV).

This work was supported by Inserm Atp 77–79.

We are grateful to Dr Y. Chardonnet for advice on virus purification and to Miss H. Linois for technical assistance.

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Recurrent genital candidosis in women and the effect of intermittent prophylactic treatment

FIONA DAVIDSON AND R. F. MOULD

From the Department of Sexually Transmitted Diseases and the Department of Medical Physics, Westminster Hospital

SUMMARY A study of clotrimazole for the treatment of recurrent genital candidosis unexpectedly showed that symptoms and infection can be dissociated. The aim of the study was to see if intermittent antifungal treatment would reduce symptoms in women constantly distressed by recurrent genital candidosis. Forty women seriously affected by the condition were initially given oral and local antifungal treatment. When the patients were symptom-free and the vagina was free of yeasts, they were entered into a double-blind clinical trial and were treated prophylactically for four months with either intermittent clotrimazole pessaries and cream or a placebo. The prophylactic treatment kept symptoms below a critical level but did not affect the return of the yeasts to the vagina. This dissociation between symptoms and vaginal yeasts was unexpected. Rectal yeast carriage was unaffected by prophylactic vaginal treatment. Male contacts and patients both showed a high incidence of non-specific genital infection. This association has seldom been reported. A few patients cultured yeasts from their homes but this environment was not considered a major source of reinfection. The vaginal pH did not appear to be altered by the presence of yeasts. The results of the study suggested that symptoms in women with recurrent genital candidosis were not caused by yeasts alone, and possibly the reason for recurrences might lie not in constant reinfection by yeasts, but in failure to recognise and remove a primary underlying factor, perhaps infection with other sexually transmitted agents. The question of a synergistic action between yeasts and other organisms is discussed.

Introduction

Yeast infection of the genital and perianal area in women is common (Hurley *et al.*, 1973; Hilton and Warnock, 1975; Willmott, 1975) and according to the report of the Chief Medical Officer for 1975 (Department of Health and Social Security, 1976) the incidence is increasing. This is said to be enhanced by such factors as antibiotics, immuno-suppressive drugs, pregnancy, and diabetes (Gardner and Kaufman, 1969), while even nylon tights have been implicated (Bull, 1969).

There is however a group of women prone to recurrent attacks of genital and perianal candidosis in whom no obvious provoking factor can be found. These patients have recurring attacks at varying intervals, often for many years causing much distress

to themselves and their sexual partners. This study was therefore undertaken to find the effect of intermittent prophylactic treatment in this group of women.

Patients and methods

Forty women attending the Westminster Hospital Department of Sexually Transmitted Diseases between 1974 and 1976 were selected for the trial. The criterion for their inclusion was that they had either a history of recurrent 'thrush' for at least one year treated elsewhere each time with antifungal therapy, and confirmed where possible, or had had at least three proved treated clinical attacks of candidosis in the previous six months in the clinic.

In all cases the patients had distressing symptoms of itching or discharge, signs of vulvitis or vaginitis (Carroll *et al.*, 1973), and yeasts were grown on culture. At each visit oral, vaginal, and rectal swabs were taken with polyester sponge swabs (Oates *et al.*, 1971). The oral swabs were taken from both sides of the buccal mucosa opposite the first molar tooth.

Presented in part as a Free Communication at the Eighth World Congress of Gynaecology and Obstetrics in Mexico, October 1976

Address for reprints: Dr F. Davidson, Westminster Hospital, Department of Sexually Transmitted Diseases, Dean Ryle Street, London SW1P 2AP

Received for publication 11 July 1977

The vaginal swab was taken from the secretions in the posterior fornix and midvaginal epithelium, and a swab was taken from the rectal mucosa through a proctoscope. Smears were stained by Gram's method and examined microscopically for yeasts, and the swabs were cultured in Feinberg-Whittington medium at 37°C for 48 hours. The yeasts cultured were further identified by the germ tube method and sugar fermentation. The pH of the vaginal discharge was measured at each visit with simple narrow range litmus paper* accurate to 0.5.

As the patients selected had to attend regularly for a year, only those who were seriously distressed by the problem, who appeared reliable, were co-operative, and lived reasonably near were included in the trial. It was hoped these precautions would reduce defaulting to the minimum. A schematic diagram of the trial is shown in Fig. 1.

All the patients were treated initially with a standard regimen, consisting of one oral nystatin tablet three times a day for 14 days, and one vaginal clotrimazole pessary each night, with clotrimazole cream to the genital and perianal area for 12 consecutive nights. The patients were instructed exactly how to use the pessaries and cream; to use them during menstruation if it occurred and without internal tampons. They were also told not to douche or wash out the vagina, use any additives to their baths, nor use any vulvovaginal deodorants.

One week after finishing this treatment the patients were re-examined. This was the assessment point for entering the trial. If symptoms had subsided and the

Gram-stained smear was negative for yeasts, while waiting for the culture result, the patient was given a code number which placed her randomly in one of the two groups. The code was held by the pharmaceutical company† which provided the drugs, and was not broken until the end of the trial.

The first group was given active clotrimazole pessaries and cream for prophylactic use. They had four special packs each containing six pessaries and one tube of cream. The second group was given similar packs of placebo pessaries and cream. The active treatment was identical in appearance with the placebo treatment, which consisted of the inactive base. None of the patients knew that there was placebo treatment. Ethical approval was given for this study by the appropriate committee.

Each patient was given an emergency pack containing six active clotrimazole pessaries and a tube of clotrimazole cream for use only if severe symptoms developed between follow-up visits and she could not attend the clinic.

The patients used the follow-up treatment of one pessary and cream each night from the fifth to eleventh day inclusive of the next four menstrual periods. They returned for examination two weeks after the end of each treatment, just before the next period was due. It was at this time that relapse was considered most likely to occur.

At each visit they were asked whether they had used the treatment or had needed the emergency

*Whatman Biochemicals
†Bayer UK Ltd

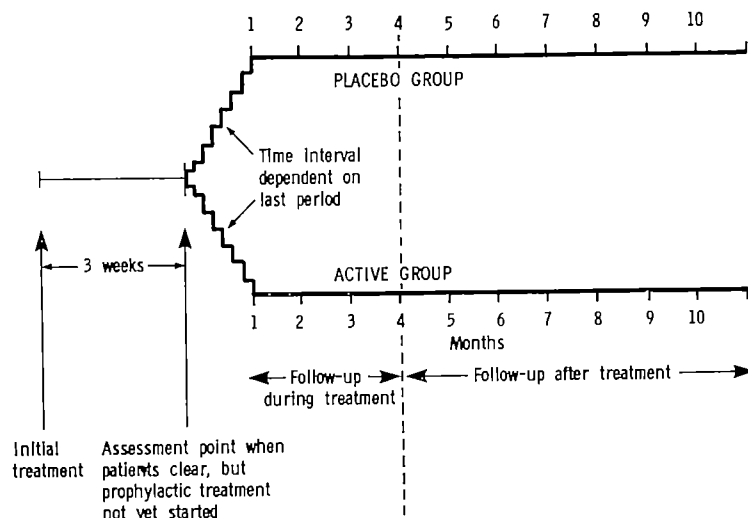


Fig. 1 Schematic diagram of the trial.

pack. At the end of the four months' treatment they continued to return monthly in the same way for a further six months.

Each patient was also given two Sabouraud-chloromycetin culture plates to expose at home, one in the bathroom and one in the bedroom, to see if *Candida* species would grow from the atmosphere.

Results

Forty women entered the trial with 20 allocated to the active treatment and 20 to the placebo treatment. Table 1a and b shows that both groups were similar in terms of age, duration of recurrent attacks of

'thrush', and duration of the presenting attack. One patient in the placebo group was diabetic.

As some of the patients had attended other clinics for previously proved attacks of 'thrush' the range of number of proved attacks treated at the Westminster Hospital clinic varied in both groups from two to 13.

SYMPTOMS

As the trial set out to evaluate the effect of a prophylactic treatment for four months, all the patients were free from severe symptoms before beginning the treatment under study. Figure 2 shows the effect of this treatment on severe symptoms for the two groups. It illustrates when each patient either

Table 1a Data for the two groups of patients in the trial

Group	No. of patients in group	Ages of patients (years)		Duration of recurrent 'thrush' (years)		Duration of presenting attack (days)	
		Mean	Range	Mean	Range	Mean	Range
Active	20	25	(20-30)	3	(0.5-9)	27	(3-140)
Placebo	20	26	(19-43)	3	(0.5-8)	29	(2-168)

Table 1b Data for the two groups of patients in the trial

Group	No. of proved attacks of yeast infection up to time of trial in clinic		No. of patients treated previously with clotrimazole	No. of patients with known non-specific infection during trial
	Mean	Range		
Active	4.2	(2-13)	17	12
Placebo	4.3	(2-13)	17	14

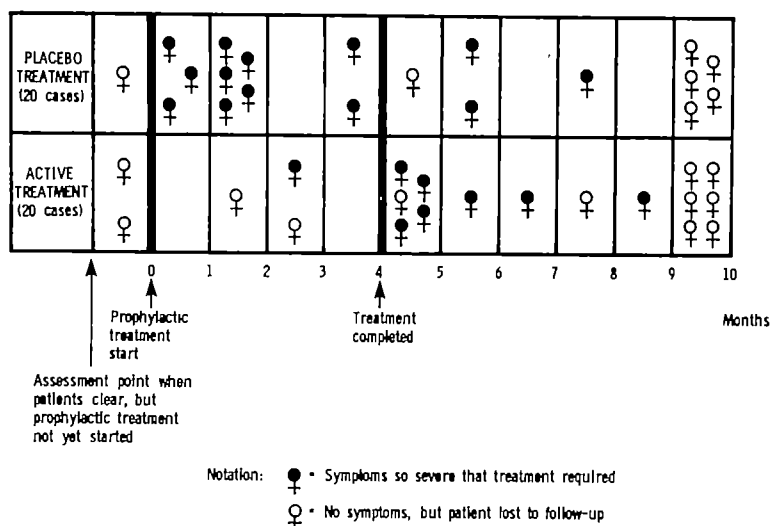


Fig. 2 Follow-up diagram of all patients in the trial, indicating when symptoms became so severe that treatment was required.

developed symptoms so severe that retreatment was required or, if she did not complete the course, when she was last seen free from symptoms. For example, in the placebo group one patient free from symptoms was 'lost' before the prophylactic treatment was started and another was last seen free from symptoms after four months. In the active group, two patients were 'lost' before treatment began and one each was last seen free from symptoms after one, two, four, and seven months. This was in spite of every possible effort made to get the patients to attend.

Symptoms have been considered only when severe enough for retreatment as terms such as itching and discharge were thought to be too subjective. Any patient who had used her emergency pack before her visit was considered to have had symptoms severe enough for retreatment even if at the next visit she was then asymptomatic, or the vagina showed no yeasts.

Because of the loss of patients before treatment, the numbers available for statistical analysis were reduced to 19 in the placebo group and 18 in the active group. The most notable feature of Fig. 2 is the pattern during the first four months. During this period 10 of the 19 patients in the placebo group demonstrated symptoms so severe that they required retreatment during this first four months but only one of the 18 patients in the active group required retreatment during this period. This is statistically highly significant, $p < 0.01$. However, the active group contained two patients lost to follow-up during this time but even if it is assumed that one of these exhibited severe symptoms before the four months elapsed, the difference between the two groups is still statistically significant (Table 2).

After prophylactic treatment stopped, the patients in the active group began to relapse and required retreatment. At the last follow-up at 10 months, five of the 19 patients in the placebo group and six of the 18 patients in the active group were still symptom-free. However, they are not directly comparable because of the different pattern of follow-up

Table 2 Contingency table relating symptoms after treatment in the placebo and active treatment groups

Group	Symptoms during 4 months' treatment	
	Severe symptoms	No severe symptoms
Placebo	10	9
Active	2	16

With Yates's correction $\chi^2 = 5.51$ $P = 0.019$.

The data for this table were obtained from Fig. 2. In the active group, 2 patients were lost to follow-up during the four months of treatment while asymptomatic. An assumption is made that on average half those lost to follow-up would have experienced severe symptoms. Thus, in the contingency table the numbers 2 and 16 refer to the active group.

losses in the two groups. Seven patients in the placebo group and one patient in the active group had used their emergency packs by the end of the first four months. Most of the emergency packs were used in the first two months of the trial, and most of the failures in the active group were in the first two months after all treatment had ceased, indicating that symptoms were kept under control by the clotrimazole.

CULTURES

Figure 3 shows the return of the yeasts to the vagina, regardless of symptoms, for the two groups of patients. There is no significant difference between the results of the two groups, $p > 0.5$, Table 3.

The presence of symptoms, and positive yeast culture results at each patient-visit were recorded (Table 4). In both groups severe symptoms occurred with negative culture results and positive results occurred without symptoms. There was no definite relationship between time to reappearance of yeasts and time to symptoms.

RECTAL YEASTS

Fewer than half of the patients with recurrent genital candidosis carried rectal yeasts initially, six out of 19 and eight out of 19 in the two groups. After oral nystatin only one of the six in the first group and two of the eight in the second group still grew yeasts. However, during the four months after oral nystatin the yeasts returned and at the end of this time the number with rectal yeasts was slightly higher than initially, eight out of 19 and 10 out of 19 in the two groups. There was no difference in the rectal yeast carriage rate during the prophylactic treatment between the active and placebo groups.

ORAL YEASTS

Thirty-two of the original 40 patients had oral yeasts before treatment and 26 of these had oral yeasts after two weeks' treatment with oral nystatin. There

Table 3 Contingency table relating regrowth of yeasts in the vagina after treatment in the placebo and active treatment groups

Group	Vaginal yeasts during 4 months' treatment	
	Yeasts regrew	No yeasts grown
Placebo	10	9
Active	9.5	7.5

With Yates's correction $\chi^2 = 0.10$ $P > 0.5$.

The data for this table were obtained from Fig. 3. In the active group, three patients were lost to follow-up during the four months of treatment while asymptomatic. An assumption is made that on average half those lost to follow-up would have regrown yeasts and half would not. Thus, in the contingency table the numbers 9.5 and 7.5 refer to the active group.

were insufficient patients either without oral yeasts or without orogenital contact to make any comparisons in this study.

CONTACTS

Information was available concerning 36 male contacts of 32 patients. Thirty-two contacts were seen in the Westminster Clinic and four contacts

were seen in other clinics. Four patients had two contacts, two patients had no contacts, and six patients had contacts who refused to attend. Twenty-four contacts had coronal and meatal swabs taken of which 10 (42%) grew yeasts, and five contacts had symptoms of balanitis but were not seen at the clinic. Thirty-three of the total 42 contacts used clotrimazole cream to reduce the possibility of

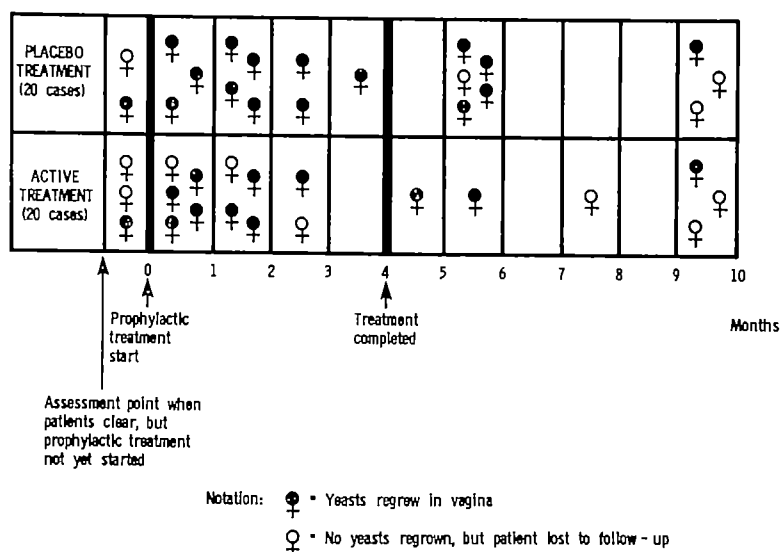


Fig. 3 Follow-up diagram of all patients in the trial, indicating when yeasts grew in the vagina.

Table 4 Results for symptoms and reappearance of yeasts

Placebo treatment			Active treatment		
Patient	Time to symptoms (months)	Time to reappearance of yeasts (months)	Patient	Time to symptoms (months)	Time to reappearance of yeasts (months)
1	0	3	1	0	1
2	0	4	2	0	1
3	0	10	3	0	2
4	1	2	4	0	10
5	1	2	5	3	0
6	1	6	6	5	1
7	2	1	7	5	1
8	2	1	8	5	2
9	2	2	9	5	5
10	2	6	10	6	2
11	2	6	11	7	6
12	4	2	12	9	3
13	4	3			
14	6	0			
15	6	6			
16	8	1			

Placebo group

One patient was lost to follow-up

Two patients had no symptoms and also yeasts did not reappear

One patient had no symptoms, but yeasts reappeared two weeks before start of placebo treatment

Active treatment group

Two patients were lost to follow-up

Five patients had no symptoms and also yeasts did not reappear

One patient had no symptoms, but yeasts reappeared two weeks before start of placebo treatment

reinfection. The other nine refused treatment. Twenty male contacts had non-specific urethritis: several were asymptomatic and in three this was associated with genital warts. A further five contacts had a history of non-specific urethritis while associating with the patients. Thus 25 (70%) of the 36 male contacts seen had non-specific genital infection during their association with the patients with recurrent genital candidosis.

Twelve (63%) patients in the active group and 14 (74%) patients in the placebo group had had contact with a patient with non-specific urethritis or were considered to have non-specific infection diagnosed from signs of cervicitis, urethritis, or salpingitis in the absence of known pathogens. Some of the male contacts of these patients however were not seen.

Thus, contacts and patients had a high incidence of non-specific genital infection.

CONTRACEPTION

Seventeen of 20 in the active group and 10 of 20 in the placebo group were taking the contraceptive pill.

VAGINAL pH

The vaginal pH as measured by the narrow range pH paper, before and after treatment in the patients with recurrent candidosis, was compared with a control group with no evidence of yeast infection. There was no difference between the groups. The vaginal pH was remarkably constant for individual patients. Variations occurred with the presence of blood or after coitus.

ENVIRONMENT

Twenty-eight of the 40 patients returned their bathroom and bedroom culture plates. Eleven of the 56 plates grew *Candida* species (five *Candida albicans* and six *Candida guilliermondii*). These organisms were isolated from the corresponding patients. No *C. albicans* was grown from a group of 21 normal controls,* only two of 42 plates grew *Candida* species, one grew *Candida parapsilosis* and the other *C. guilliermondii*.

YEAST IDENTIFICATION

Thirty-two of the 40 patients grew *C. albicans* from the vagina, five of these with *C. guilliermondii* or *Torulopsis glabrata*. When yeasts were isolated from mouth, vagina, and rectum, *C. albicans* was usually grown, although occasionally the cultures from these sites differed from each other. The yeasts in the remaining eight patients were not identified.

*Normal controls were culture plates taken home by staff from the clinic. These people had no known complaints but were not examined

Discussion

Odds (1977) remarked that an antifungal preparation should be expected to eliminate only the yeasts from the female genital tract. Its success in relieving symptoms depends on those symptoms being caused exclusively by the yeasts. In the trial of antifungal treatment it was expected that the reduction in symptoms would be associated with the reduction in yeasts cultured from the vagina. Therefore, the observed dissociation between symptoms and positive yeast cultures was a surprise. The intermittent clotrimazole treatment undoubtedly reduced symptoms in the patients with recurrent genital candidosis, which was the original aim, but therapy did not affect regrowth of yeasts in the vagina. It could be argued that symptoms were associated with the number of yeast colonies present and that intermittent treatment simply reduced the total colony count below a critical level. However, Mursic (1975) found that patients with or without symptoms could have the same yeast count.

One possibility is that clotrimazole was acting locally on some factor other than the fungus alone. When the treatment stopped, the symptoms returned in many patients but were not always accompanied by the fungus. Mendel and Haberman (1965) commented on this aspect. They studied patients with cultural and clinical cures who returned later with recurrent symptoms, although the cultures were often still negative. They noticed that clinical cures and alleviation of symptoms occurred during therapy when the organisms were still present, and that in some cases the same fungal treatment could relieve those with symptoms in whom no *Candida* was ever isolated.

These observations suggest *Candida* is always a secondary invader in the vagina, either combining with an underlying factor to produce symptoms or enhancing the symptoms this factor has already produced. Gardner and Kaufman (1969) referred to this underlying factor as Factor X.

C. albicans as a primary infecting organism does not really fulfil Koch's postulates (Koch, 1890). It can occur in the vagina without signs or symptoms of disease and the classical signs and symptoms of candidal vaginitis may occur without the isolation of the yeasts (Carroll *et al.*, 1973). Maibach and Kligman (1962) found it impossible to infect a healthy subject with *Candida*. Damage was always needed first.

However, one of the most striking features among the patients in the trial and their contacts was the high incidence of non-specific infection. Seventy per cent of the male contacts had non-specific infection during their association with the patients

in the trial. In several cases the male contacts of the trial patients were asymptomatic and the urethritis would have been missed if not looked for carefully. A high proportion of the men carried yeasts on the penis confirming earlier results (Davidson, 1977). Rohatiner (1966) found a similar association with non-specific urethritis and he commented on the possibility of a relationship between *Candida* and other organisms found in the male or female genital tract. Loh and Baker (1955) while investigating the effect of tetracycline on yeasts in the gut noticed there was a longer and more persistent overgrowth with *Proteus* species. Then Yamabayashi (1958) noticed that *C. albicans* enhanced the virulence of *Proteus vulgaris*, *Pseudomonas aeruginosa*, and to a lesser extent *Escherichia coli* in experimental mice but it did not enhance the growth of these organisms *in vitro*, nor did it affect the virulence or growth of *Staphylococcus aureus*, *Bacillus subtilis*, or *Streptococcus haemolyticus*. Mendel and Haberman (1965) noticed a strong correlation, in their patients, between positive *Candida* cultures and the presence of *E. coli* and *A. aerogenes* in the vagina. Söltz-Szöts and Thurner (1969) said pure cultures of *Candida* were almost never found in the male urethra. They considered that *Candida* could only multiply in the urethra if, together with the *Candida*, there was irritation of the mucosa by trauma, bacteria, viruses, or *Trichomonas vaginalis*. They also noted candidal balanitis was often associated with massive bacterial colonisation. Willmott (1975) noticed 34% of his patients with yeasts had other infections.

The possible association of yeasts with non-specific genital infection produces many problems. The causes of non-specific infection are as difficult to define as the causes of candidal vaginitis. The most persistently implicated aetiological agents in non-specific infection are *Chlamydia trachomatis* and the mycoplasmas but no pathognomic signs or symptoms need occur in either sex, and they may be asymptomatic.

The possibility of reinfection of the vagina with yeasts from the rectum raises another unanswered question. There is a known association between *Candida* of the rectum and *Candida* of the vagina (Anyon *et al.*, 1971), but this does not necessarily infer a causal relationship between them. Not all the patients in the trial had yeasts in the rectum and certainly the results showed no evidence that the vagina was infected from the rectum or vice versa. *Candida* was found to occur in the homes of a few of the patients but the environment was not considered a major contributory factor in these recurrences. The acidity of the vagina in those patients with recurrent genital candidosis was remarkably constant and did not differ from those with no

yeast problem. Similar results were found by Cohen (1969).

More patients in the active treatment group than the placebo group used oral contraceptives, but as yeast regrowth was the same in both groups and the symptoms were less in the active group, there is no evidence that oral contraceptives had any marked influence on these patients.

It has long been stated that *Candida* is a disease of the diseased. Wilson (1962) believed that it could even indicate underlying disease before this was clinically detectable. Kirkpatrick *et al.* (1971) considered that treatment directed only to the organism could be predictably inadequate since the 'fertile soil' for *Candida* persisted in deficient hosts even after elimination of the organism. As a consequence, although the remission with chemotherapy might be complete, it was almost always temporary. The rational approach should be towards the host and not the organism.

It may be that the common factor necessary is some disorganisation of skin or mucous membrane at a cellular level which allows the fungus to become pathogenic. Simple remedies such as preventing self damage might be sufficient—for example, no douching, no vaginal deodorants, no bubble baths. Perhaps the use of simple lubricants such as KY Jelly* in sexual intercourse for those who do not lubricate well, might prevent mechanical damage. However it is most important to consider always the possibility of underlying infections in women with recurrent genital candidosis and in their contacts.

We are very grateful to Dr J. K. Oates for permission to investigate his patients, and to Mr N. Richardson and Mr M. Y. Imambaccus for their expert technical assistance. We would like to thank Bayer Pharmaceuticals (UK) Ltd for their assistance and for supplying clotrimazole. We are also grateful to Mrs V. S. Chapman for secretarial help.

*Johnson and Johnson Ltd

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Clotrimazole (Canesten)* in the treatment of candidal balanitis in men

With incidental observations on diabetic candidal balanoposthitis

M. A. WAUGH,† E. G. V. EVANS,‡ K. C. NAYYAR,† AND R. FONG†

From the Department of Genitourinary Medicine† and the Unit of Mycology (dermatology and medical microbiology),‡ General Infirmary, Leeds

SUMMARY Clotrimazole 1% (Canesten) cream was used in a trial comprising 138 men with candidal balanitis. Eighty-six (91%) out of 94 men were asymptomatic after seven days and 57 (98%) of 58 men were asymptomatic after three weeks' treatment. After seven days, *Candida albicans* could no longer be cultured from 86 (90%) of 96 men, nor from 55 (95%) of 58 men three weeks after treatment. Microscopical examination gave varying results. Fifteen (10.9%) of 138 men were found to have diabetes mellitus; this group was significantly older than the non-diabetics. It is concluded that treatment with clotrimazole for seven days is effective in the treatment of candidal balanitis.

Introduction

In recent years, the importance of genital candidosis in men has been stressed. The patient with genital yeasts may be asymptomatic, but such yeasts are found if a diligent search is made in sex partners of women with vaginal candidosis. The male may complain of burning and itching of the penis which will develop within hours of sexual intercourse, and a generalised erythema of the glans and prepuce may be noted (Oates, 1976). This may be caused by hypersensitivity to *Candida albicans*.

In other patients symptoms are less acute. Perhaps a complaint is made of some redness of the glans and prepuce, and there is a whitish subpreputial discharge. The typical case is mild, with a dry, glazed, erythematous surface to the glans penis and on the undersurface of the prepuce. In addition, there may be very small, irregular, eroded papules and a dispersed vesicular eruption, often with plaques of white cheesy matter. The fossa navicularis may be involved and there may be a transient urethritis. Itchy and scaly erythematous lesions may also be present on the penile and scrotal skin, and in the

obese, the groins may be involved. An acute fulminating oedematous type of balanoposthitis may occur with ulceration of the penis and a swollen fissured prepuce. This is often associated with diabetes mellitus.

Clotrimazole is a tritylimidazole derivative which acts on the cell membrane. Iwata *et al.* (1973) suggested that fungicidal concentrations of clotrimazole damaged the permeable membrane allowing leakage of intracellular phosphorus compounds and potassium ions, leading to inhibition of macromolecular synthesis. The drug is active *in vitro* against *C. albicans*, most strains being inhibited by 2 mg/l or less, and killed by less than 10 mg/l of the drug (Holt, 1974). It is available as a cream for topical treatment.

Material and methods

An uncontrolled trial was conducted to study the cure rate of patients with candidal balanitis or balanoposthitis and treated with 1% clotrimazole (Canesten) cream.

The 138 patients studied were treated between 1 January 1976 and 30 April 1977. They were included only if culture of *C. albicans* on Sabouraud's medium confirmed the initial findings of mycelia or if spores were found by microscopical examination of Gram-stained material taken from the glans penis, coronal sulcus, or subpreputial sac.

*Canesten is the trade mark of Bayer (UK) Ltd

Address for reprints: M. A. Waugh, The General Infirmary, Department of Genitourinary Medicine, Leeds LS1 3EX

Received for publication 1 August 1977

Read as a paper at the MSSVD Spring Meeting, in Vienna 7 May 1977

Two dry cottonwool swabs were taken from the coronal sulcus. A smear was made from the first, stained by Gram's method, examined microscopically using a $\times 100$ objective, and sent to the mycology unit for confirmation. The second swab was inoculated on to Sabouraud's culture medium in the clinic and sent on to the mycology laboratory, where it was examined by microscopy and Sabouraud's culture for *C. albicans*.

Urine from all patients was tested using clinistix (Ames) and all patients with glycosuria had an oral glucose tolerance test.

On the initial visit, note was taken of the duration of balanitis; previous treatment had to have stopped at least seven days earlier if the patient was to be included in the trial. Note was made of concurrent oral antibiotics, corticosteroids, or other treatment.

The patient was asked if the sex partner had vaginal irritation, discharge, or was being treated for a vaginal condition, and if so, did he know her diagnosis. Subsequently, the case notes of these sex partners confirmed this and gave more clinical information.

On the initial visit, note was made of the area affected—glans or prepuce, shaft, scrotum, or groin.

Observations were to be made on the patient before treatment, at the end of the first week of treatment, three weeks after the end of treatment, and at long-term follow-up (mean 9.23 weeks, SD 6.10, s.e.m. 1.36). On each visit, symptoms of irritation, if present, were tabulated. Swabs were collected and examined as before.

After the investigations in the clinic had given positive results the patient was told to apply clotrimazole 1% cream gently, each morning and at night for seven days, washing off the cream the night before attending the clinic for the follow-up visit.

At the end of one week of treatment, the patient was asked if he had benefited from treatment at all and his answers were noted on a graded reply chart. He was asked if he had completed treatment, had had sexual intercourse since starting treatment, and if treatment had upset him in any way (graded reply chart). The clinical impression was also recorded using the following grades—cured, improved, no change, worse, or don't know.

Results

CLINICAL DATA

One hundred and thirty-eight patients (age range 16–68 years, mean 27.7, SD 9.9, s.e.m. 0.8) were admitted to the trial, but 39 (28.2%) (mean 28 years, SD 9.6, s.e.m. 1.5) defaulted after the initial visit. Therefore, 99 patients completed the study.

Associated genital conditions were nongonococcal urethritis in 26 (18.8%) patients, condylomata acuminata in six (4.4%), trichomoniasis in three (2.2%), gonorrhoea in two (1.4%), Reiter's syndrome in one, and balanitis xerotica obliterans in one.

Concurrent treatment was prescribed in 34 patients, oral antibiotics in 20 (14.5%), oral corticosteroids in three (2.2%), and other medication in 11 (8%) patients.

AREA AFFECTED

Of 138 men in the trial, the glans or prepuce was affected in 137 (99.3%), the shaft of the penis in 17 (12.3%), groin in four (2.9%), and the scrotum in two (1.4%) patients.

DETAILS OF SEX PARTNER

Vaginal irritation was present in 41 (29.7%) of the sex partners, and vaginal discharge in 39 (28.3%). A diagnosis of vaginitis was made in 33 (23.9%) women and in 29 (21%) of these this was caused by candidosis. Vaginal treatment was given to 33 of the sex partners for whom details were obtained. Twenty-nine were treated for candidosis, 22 with clotrimazole and seven with nystatin.

Results

TREATMENT OF CANDIDAL BALANITIS WITH CLOTRIMAZOLE 1% CREAM

Final analysis was on the results for 99 patients who completed the study. Symptoms had gone in 89 (93%) of 96 after seven days of treatment. Of those followed up three weeks after the end of treatment 57 (98%) of 58 were asymptomatic and on long-term follow-up, 18 (90%) of 20 remained so. There were no serious side effects.

Yeast cells could no longer be seen by microscopical examination after seven days in 69 (73%) of patients, three weeks after the end of treatment in 43 (75%), and at long-term follow-up in 14 (74%) of patients. In no cases were mycelia found after treatment. They were found in only 54 (59%) before treatment, on initial microscopy.

After culture on Sabouraud's medium 86 (91%) of 94 gave negative results after one week, 55 (95%) of 58 three weeks after the end of treatment, and 18 (90%) of 20 on long-term follow-up.

Eight (8%) of 94 cultures still gave positive results at the end of one week, two (4%) of 58 three weeks after the end of treatment, and two (10%) of 20 on long-term follow-up.

Treatment of the symptoms of candidal balanitis using clotrimazole 1% cream was successful in 93% of patients, and follow-up negative cultures for

C. albicans varied from 91% after one week to 96% of those patients followed up three weeks after the end of treatment.

Associated study on diabetes mellitus presenting with candidal balanitis/balanoposthitis

Of 138 patients with candidal balanoposthitis 15 (10.9%) were found to be diabetic. Their ages ranged from 19–68 years, mean 39.4, SD 17.8, s.e.m. 4.6. Two defaulted after the initial visit but six were in the group that was followed up the longest. These patients were found to be significantly older than non-diabetics presenting with candidal balanitis/balanoposthitis ($t=2.833$, $P<0.02$). In addition two patients were found to have renal glycosuria.

After one week of treatment the diabetic patients had a higher proportion of positive culture results (four of 13) than the non-diabetics (four of 81) ($\chi^2_1=6.565$, $P<0.01$).

During the period of the study a total of 6971 new cases were diagnosed in men, but only six diabetics were found among men who were not in the study.

Discussion

Estimates of the infectivity of *C. albicans* to the male have varied from about 10% (Oriel *et al.*, 1972; Morton and Rashid, 1977) to 33% by smear or culture or to 49% by culture only (Willmott, 1975). Considering that many women are treated by other doctors for candidal vaginitis the infectivity rate of the female is probably much higher than is realised. As vaginal candidosis is the infective gynaecological condition that is most commonly seen, investigation and treatment of the male partner becomes important if this distressing condition is to be contained.

Unfortunately the motivation for follow-up disappears fairly rapidly once the patient realises he has had a relatively benign complaint and has got over his early anxiety of an abnormal genital condition. The default rate was similar to that found by Masterton *et al.* (1975) when treating genital candidosis with natamycin cream.

On the other hand, it could be argued that treatment which has considerable success even when given for such a short time as a week may have beneficial results in preventing the transmission of candidosis in sexually active patients.

Fifteen (10.9%) of 138 patients were found to have diabetes mellitus. Diabetic candidal balanitis occurred in an older age group and, in the uncircumcised male, balanoposthitis presented with an oedematous fissured prepuce which in some cases had become almost impossible to retract. There were often symptoms of vague ill health preceding this for a few months, rather than the characteristic well-known presenting symptoms of diabetes mellitus.

After treatment, yeasts could be seen on microscopical examination while none grew on culture. An explanation for this may be that the yeasts were no longer viable. In this trial, clotrimazole had no serious side effects such as a sensitivity reaction. It was acceptable to the patient even when he persisted in his sexual activities while undergoing treatment. It did not stain bed clothes or underwear.

We should like to thank Bayer UK Limited for help in presenting this study and Mr J. Bailey, statistician to Bayer UK Limited, for his help in analysing the results.

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Measuring the outcome of contact tracing

1: A description of the patient and contact populations studied

ANN SATIN AND ANGELA MILLS

From the Health Education Council, London

SUMMARY A standard system for health workers to record and handle information on patients and contacts was introduced to five clinics. Information on all the patients interviewed during the first quarter of 1975 was collected and analysed. An analysis of the characteristics of these patients showed variation between the clinics. However, regardless of what was being measured, there were always differences between men and women. It was found that not all infected patients were interviewed about their contacts. Unless or until contact tracing includes all infected patients, the effect of contact tracing on the control of the sexually transmitted diseases will be of limited value and difficult to assess.

Introduction

Measurement of the effectiveness of contact tracing in the control of sexually transmitted disease is complicated by the difficulty of determining the individuals comprising the population to be investigated. The object of contact tracing is to ensure that all individuals who have had sexual contacts with infected persons seek examination and are treated if necessary. The base population is, therefore, clinic patients known to be infected. A Health Education Council (HEC) project, was begun in 1972 to establish criteria by which effectiveness can be assessed (Mills and Satin, 1976).

A standard system for recording and handling information on the contacts of patients found to have venereal diseases was recommended and introduced to five clinics (Satin, 1977). Information recorded by health workers at these clinics was collected and used to assess results of the contact investigations. This paper describes the patient and contact populations which were the subjects of investigation. It is followed by an evaluation of the outcome of the contact tracing investigations (Mills and Satin, 1978).

Method

The data for this study were extracted from the information recorded by 12 health workers on the

Address for reprints: A. Mills, 47 Albany Mansions, Albert Bridge Road, London SW11

Received for publication 12 December 1977

separate documents for patients and contacts which comprise the HEC record system. There was no attempt to evaluate the individual effectiveness of the health workers at the five clinics. Four of the clinics are located in London; the fifth is in Oxford* (Satin, 1977).

The information recorded on the separate documents was coded and transferred to punch cards for analysis. The original records remained in the clinic of origin. For the analysis the computer program and computer time were kindly provided by the DHSS computer centre at New Court, Lincoln's Inn Fields.

The study population was divided into two categories: (1) those patients interviewed by health workers during the period from 1 January to 31 March 1975, and (2) the contacts (sexual partners) named by the patients interviewed. All patients had been diagnosed and found to have gonorrhoea or infectious syphilis, or both.

Results

The number of patients interviewed during the period at each clinic was compared with the number of cases of gonorrhoea and syphilis diagnosed during the same period and reported to the DHSS.

*The clinics taking part were at University College Hospital, London (UCH); St Giles Hospital, London; Middlesex Hospital, London (JPH); St Thomas' Hospital, London (STH); Radcliffe Infirmary, Oxford.

The difference between the two sets of figures, with one exception, reveals that health workers did not fulfil the expectation of interviewing all diagnosed patients about their contacts.

The total number of contacts named by patients and recorded by health workers comprises the contact population. The number of patients and contacts recorded is presented in Table 2. A total of 1379 infected patients named 2088 contacts.

Table 3 presents the percentage of patients who named no contact, or one or more. More than 50% of both male and female patients at four of the five clinics named only one contact.

Table 1 *Patients diagnosed 1 January–31 March 1975: interviews recorded compared with cases reported*

Clinic	Gender of patients	Total interviewed (recorded by health workers)	Total reported (from DHSS records)
UCH	Male	182	185
	Female	88	96
St Giles	Male	133	153
	Female	76	89
JPH	Male	295	317
	Female	96	117
Radcliffe	Male	59	65
	Female	50	54
STH	Male	255	255
	Female	145	130

Table 2 *Total number of patient and contact records*

	UCH	St Giles	JPH	Radcliffe	STH	Totals
Patients						
Male	182	133	295	59	255	924
Female	88	76	96	50	145	455
Total	270	209	391	109	400	1379
Contacts						
Of male patients	277	201	425	110	425	1438
Of female patients	122	105	139	98	186	650
Total	399	306	564	208	611	2088

Table 3 *Percentage of patients naming one, two, or more contacts*

Clinic	Gender of patients	Percentage of patients naming contacts				Total no. of patients
		None	1 contact	2 contacts	3 contacts or more	
UCH	Male	3	64	26	7	182
	Female	—	71	27	2	88
St Giles	Male	2	56	34	11	133
	Female	3	60	32	4	76
JPH	Male	1	67	24	7	295
	Female	—	67	25	8	96
Radcliffe	Male	—	47	37	16	59
	Female	—	40	38	22	50
STH	Male	1	50	39	10	255
	Female	—	77	20	3	145

The sexual orientation of patients presented in Table 4 was determined by the gender of the contact for each incident of infection. A patient was classified as heterosexual if contacts were of the opposite sex, homosexual if contacts were of same sex, bisexual if both male and female contacts were named.

The number of contacts recorded for each patient is presented in Table 5. The contact index in this table is the mean number of contacts named by patients at interview. The contact index was determined by dividing the number of contacts by the number of patients naming those contacts. Two clinics had comparatively high numbers of male patients naming male contacts. For this reason the contact index takes into account the gender of the patient and that of the contact. However, it should be noted that, as male bisexual patients named both male and female partners, they are represented twice in the table, in both of the rows relating to male patients.

The age of each patient was determined by the recorded date of birth. Excluding pre-pubertal cases (not interviewed by health workers), individuals under 16 years represent less than 1% of the total; three of 904 male patients, and eight of 441 female patients were in this category. There were no boys under 16 treated at UCH, JPH, or the Radcliffe. No girls under 16 were treated at JPH.

Male patients under 20 years of age accounted for only 9% of the total male patient population, in contrast to the number of females under 20 who made up 30% of the females treated. The proportion of females under 20 at the Radcliffe, 43%, was strikingly greater than at the four London clinics—JPH with 23%, UCH with 25%, STH with 30%, and St Giles with 31%. The modal age of female patients at the five clinics was 19. For males the pattern was bi-modal, peaking at 23 and 26 years. The mean age for men was 27.8, for women 23.1.

Table 6 shows differences between the sexes by age.

An analysis of age and sexual orientation of male patients was carried out. More heterosexual patients were shown to be infected at an earlier age than homosexual ones, and this finding was consistent for all five clinics. The pattern was found also in those clinics with the highest proportion of male homosexual patients. Of the 222 male homosexuals, six were under the age of 18, and 25 under the age of 21 years.

Table 7 shows that most patients, men and women, were recorded as single. UCH had proportionately more single male patients than any other clinic, and fewer who were recorded as separated or divorced. St Giles and STH, which are close to each other

Table 4 Sexual orientation of patients based on gender of partner

Sexual orientation	UCH		St Giles		JPH		Radcliffe		STH		Total	
	Male (%)	Female (%)	Male (%)	Female (%)	Male (%)	Female (%)	Male (%)	Female (%)	Male (%)	Female (%)	Male (%)	Female (%)
Heterosexual	69	99	83	99	58	100	85	100	82	99	72	99
Homosexual	27	1	9	—	39	—	10	—	16	1	24	1
Bisexual	—	—	2	—	2	—	2	—	2	1	2	1
Not recorded	4	—	6	1	1	—	3	—	—	—	2	1
Total number	182	88	133	76	295	96	59	50	255	145	924	455

Table 5 Mean number of contacts (contact index) named by clinic patients

Gender of contact and patient	UCH		St Giles		JPH		Radcliffe		STH	
	No. of contacts	Contact index	No. of contacts	Contact index	No. of contacts	Contact index	No. of contacts	Contact index	No. of contacts	Contact index
Male contacts										
Of male patients	92	1.84	28	2.00	192	1.59	13	1.86	94	2.00
Of female patients	120	1.38	103	1.37	138	1.43	97	1.94	180	1.26
Female contacts										
Of male patients	184	1.47	153	1.35	229	1.88	96	1.88	330	1.54
Of female patients	2	—	1	—	1	—	1	—	5	—
Gender of patient not recorded	1	—	21	—	1	—	1	—	2	—

Table 6 Comparison of the clinics by age of patient

Age group (years)	UCH		St Giles		JPH		Radcliffe		STH		Total	
	Males (%)	Females (%)	Males (%)	Females (%)	Males (%)	Females (%)	Males (%)	Females (%)	Males (%)	Females (%)	Males (%)	Females (%)
13-19	7	25	13	31	4	26	18	43	13	30	9	30
20-24	28	41	31	41	29	39	40	33	32	37	30	38
25-29	30	27	18	18	34	24	21	10	25	18	28	20
30-34	16	5	19	5	16	10	7	6	15	8	16	7
35-39	10	2	7	3	9	1	7	4	7	4	8	3
40-44	3	—	8	—	4	—	5	—	4	1	4	1
45-49	4	—	2	1	3	—	—	4	3	1	3	1
50+	2	—	2	1	1	—	2	—	2	—	2	1

geographically and appear to have similar patient populations, had more married female patients than the other three clinics. The Radcliffe had pro-

portionately more women recorded as separated or divorced.

Table 8 shows that there is little variation in employment patterns of patients between clinics.

Table 7 Marital states of patients

Clinic	Gender of patient	Percentage of patients			Total no. of patients
		Single	Married	Not recorded/separated/divorced/widowed	
UCH	Male	86	13	1	182
	Female	83	8	9	88
St Giles	Male	72	22	6	133
	Female	70	18	12	76
JPH	Male	76	14	10	295
	Female	89	8	3	96
Radcliffe	Male	68	20	12	59
	Female	70	10	20	50
STH	Male	74	21	5	255
	Female	67	21	12	145

Table 8 Employment state of patients

Clinic	Gender of patient	Percentage of patients				Total no. of patients
		Employed	Unemployed	Student	Not recorded	
UCH	Male	82	2	13	3	182
	Female	66	11	19	4	88
St Giles	Male	87	3	7	3	133
	Female	57	32	6	5	76
JPH	Male	81	2	12	5	295
	Female	63	9	21	7	96
Radcliffe	Male	85	5	5	5	59
	Female	62	18	16	4	50
STH	Male	90	3	6	1	255
	Female	66	30	4	—	145

Discussion

The number of patients diagnosed at the clinic determines the parameters of the population of contacts to be investigated. Variation between clinics in achieving the goal of interviewing all diagnosed patients about contacts is documented in Table 1. The evidence that health workers at one clinic were able to interview all diagnosed patients shows that it is possible to achieve that goal. The reasons offered in explanation for failure to interview all patients for the purpose of naming contacts are that:

1. Clinic staff forget or neglect to refer patients.
2. A patient leaves the clinic before interview or otherwise refuses the interview.
3. A doctor chooses not to refer a patient, having decided that the patient has had no sexual partners other than the one who transmitted the infection and should be protected from understanding the implications of the sexual transmission of his/her infection.
4. A health worker is not available owing to understaffing or poor staff management.

A perennial issue in the control of sexually transmitted diseases is the question of how closely the number of contacts identified and recorded (Table 2) reflects the actual number of contacts. The recorded number of contacts is almost certainly an underestimate of the actual contact population. Health workers taking part in this study questioned the instruction to record contact information identifying contacts whom they presumed would be untraceable. It is important to record even the slightest information about each contact in order to arrive at a more accurate estimate of the size of the infected population, indeed, some contacts initially assessed as untraceable subsequently attend a clinic. It was not possible to determine whether the differences between clinic location, clinic opening times, and extent of the catchment area served by each clinic influenced the number of contacts named.

Table 4 reveals differences between the five clinics in the sexual orientation of the patients. The finding that a markedly higher proportion of patients were male homosexuals at UCH and JPH agrees with the findings of the British Cooperative Clinical Group (1973) who found that more homosexually acquired infections were treated in London's West End clinics than in other clinics inside or outside London.

Table 5 shows that the index for male patients naming male partners was consistently higher than the index for those naming female contacts. This was true for each clinic, although it is interesting to note that the lowest contact index was found in the clinic

with the highest proportion of male homosexual patients. In three clinics, males named more female contacts than females named males. At St Giles males and females named about equal numbers and, at the Radcliffe, females named rather more male contacts than males did females. Interpretation of these findings presents problems as it is not possible to determine at the present time whether the variation in indices between clinics is owing to differences in clinic populations, differences in interviewing skill or approach among health workers themselves, or differences in approach of other clinic staff, or to a combination of factors.

Women patients were found to be infected at an earlier age than men, and fewer women were in the older age groups. These findings are strikingly similar to those cited in the report of the Chief Medical Officer (Department of Health and Social Security, 1975). For the first six months of 1975, it was found that boys under 20 years of age accounted for only 12% of the males treated for gonorrhoea. In contrast, the number of girls under 20 made up 34% of all the females treated for gonorrhoea. Fifty-five per cent of the total male patients were aged 25 or over, compared with only 30% of the total female population. It can be concluded that girls under the age of 20 are more likely to be represented in infected populations than boys under 20. This may mean that women who become infected have sexual intercourse at an earlier age than men who become infected. The data suggest that women under the age of 20 are infected by men over the age of 20.

Information on the marital and employment states of patients can be useful in contact linking and patient location. Marital state refers to a legal status, but indicates nothing about cohabiting circumstances. Some patients who are divorced or separated refer to themselves as single, not deliberately to deceive, but because this describes their domestic situation. The data presented on state of employment is of limited reliability because health workers often did not distinguish between employment state and occupation. The higher proportion of students in the clinic population at UCH, JPH, and Radcliffe reflect the location of those clinics in university areas.

Regardless of what was being measured, the following differences were observed between men and women:

1. More men than women were diagnosed and subsequently interviewed;
2. More contacts were named by men than by women, for the total population. However, the exceptions—St Giles where numbers were equivalent and the Radcliffe where women

named more contacts than men—challenge the common assumption that men have more sexual partners than women;

3. Women were more likely to be infected at an earlier age than men, but far fewer women were represented in the older age groups. These findings have implications of high or special at-risk groups.

The use of a standard recording system has made it possible to collect and compare information on clinic patients and their contacts. The results show that not all infected patients are interviewed about their contacts. Unless or until contact tracing is a service which includes all infected patients, the effect of contact tracing in the control of sexually transmitted diseases will be of limited value and difficult to measure and assess.

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Measuring the outcome of contact tracing

2: The responsibilities of the health worker and the outcome of contact investigations

ANGELA MILLS AND ANN SATIN

From the Health Education Council, London

SUMMARY The previous paper (Satin and Mills, 1978) concerned information on patients who were interviewed by health workers in five selected clinics*; information on the contacts named by these patients was similarly analysed. This paper describes and evaluates the activities of the health workers and the outcome of contact investigations. At all stages of the contact tracing process, differences were observed between men and women. It was found that more than half of the male contacts and half the female contacts were known to have been examined. Of those contacts examined, at least two-thirds of them attended the same clinic as the patient who had named them, and 85% of these contacts were found to have a treatable condition. It will be seen that the concept of success or effectiveness of contact tracing is complex in definition and measurement.

Introduction

A Health Education Council project that studied the effectiveness of contact tracing was described in the previous paper (Satin and Mills, 1978). Information collected by health workers in five selected clinics,* using a standardised system, was used to explain the extent and result of the contact investigations. The earlier paper described the patient and contact populations that were the subjects of investigation. This paper describes and evaluates the activities of the health workers and the outcome of the contact investigations. It has been found that definition and measurement of success or effectiveness of contact tracing is complex. It will be demonstrated that most patients understand the importance of informing sex partners about the risk of infection, and do so, but the influence of the health worker on this exchange is impossible to determine. As before, differences in the findings between clinics are difficult to explain. As some information was found to be missing or inaccurately recorded, it was not possible to collect complete

data from each clinic; thus some of the totals do not correspond with those in the previous paper.

Method

The methods used to collect and assess the data have already been described (Satin and Mills, 1978)

Results and discussion

HEALTH WORKER RESPONSIBILITIES

Health workers in the five clinics participating in the study were expected to interview patients and visit contacts as part of their routine case finding activities and it is these activities that were recorded and compared.

Interviews

Of the total number of patients attending clinics only 15-25% are found to have gonorrhoea or syphilis; these should be interviewed by the health worker. It is the usual practice for the health worker to interview patients and record contact identifying and locating information only *after* diagnosis of gonorrhoea or syphilis has been made and treatment given. It is assumed that the contact tracing interview will help the patient to identify partners at risk so that their prompt attendance for examination and treatment may stop the spread of infection and onset of complications. Therefore it is important to record the time intervals between patient attendance,

*The clinics taking part were at University College Hospital, London (UCH); St Giles Hospital, London; Middlesex Hospital, London (JPH); St Thomas' Hospital, London (STH); Radcliffe Infirmary, Oxford.

Address for reprints: A. Mills, 47 Albany Mansions, Albert Bridge Road, London SW11

Received for publication 12 December 1977

contact tracing interview, and contact attendance before investigating if and how these intervals can be shortened.

Table 1 shows the interval in days between the time at which patients first attended the clinic for medical investigation and the time when they were first interviewed by the health worker, by diagnosis.

Table 2 shows the same interval, but the patients are divided according to gender, and the number of patients interviewed is recorded as a cumulative percentage.

It can be seen that for a high proportion of all patients, treatment and the first interview with the health worker took place during the first attendance at the clinic. A longer interval between attendance and interview for those patients with syphilis may partly be attributed to delay in establishing a diagnosis. The higher proportion of men diagnosed and treated at first attendance was to be expected and was similar for all clinics. The St Giles clinic stands out as having the earliest completion of contact tracing interviews. This difference reflects a policy of interviewing and recording information about patients *before* they are diagnosed but when their contacts are known to be infected.

Reinterviews

Table 3 shows the percentage of patients who were interviewed by the health worker on only one occasion and those who were interviewed once, twice, or three times more.

With the exception of one clinic, there were fewer women than men reinterviewed. The policy at STH was to reinterview *all* patients who reattended, and this is reflected in the significantly higher percentage of patients reinterviewed. However, there may be several reasons why patients were not reinterviewed.

(1) They may not have reattended. (2) If at first interview diagnosis was deliberately withheld from the patient, conducting a reinterview might arouse suspicion. (3) The health worker may have asked to see that patient again but the referral not been made by the clinic staff. (4) If at first interview the health worker decided that there was nothing to be gained from seeing the patient again, then the health worker would not ask that the patient be referred. (5) There may not have been a health worker available to reinterview the patient.

Visits

Health workers visit contacts who otherwise would not be told that they needed medical care. Table 4 enables a comparison to be made between the total number of contacts named and contacts who were visited for this purpose.

The popular view that health workers spend much of their time visiting contacts is contradicted by these findings. Considering the small number of visits made by any health worker, the setting aside of a full day weekly for visiting seems difficult to justify. Rather, any necessary visit should be made with minimal delay.

CONTACT INVESTIGATION

Although for recording purposes the documents for contacts and patients are separate, it is the linkage and overlap which is a measure of successful contact investigation. Ideally, each individual involved in an infection will be (1) identified and recorded as a contact, (2) examined and treated for infection if necessary, and (3) identified by the health worker as having attended for investigation and, with patient status established, interviewed in turn for contacts.

Table 1 *Percentage of patients interviewed and their diagnoses, by number of days interviewed after attendance*

Clinic	Diagnosis of patient	Days						% for whom date not recorded or inaccurate	Total number
		Same	1-3	4-7	8-14	15-21	22+		
UCH	Gonorrhoea	63	5	9	6	1	2	14	258
	Syphilis/both infections	33	—	—	33*	—	34	—	12
St Giles	Gonorrhoea	95	1	3	1	1	—	—	195
	Syphilis/both infections	100	—	—	—	—	—	—	11
JPH	Gonorrhoea	76	2	10	6	1	3	2	341
	Syphilis/both infections	50*	—	7	5*	20	8	5*	40
Radcliffe	Gonorrhoea	76	3	11	3	2	2	—	109
	Syphilis/both infections	—	—	—	—	—	—	—	—
STH	Gonorrhoea	81	8	6	4	1	1	—	375
	Syphilis/both infections	50*	4	4	4	17*	21	—	24

*Includes those patients who were diagnosed as having both gonorrhoea and syphilis

Table 2 *Interval between attendance and interview, by gender of patient (cumulative percentage)*

Clinic	No. of patients	Percentage interviewed				
		Same day	Later (days)			
			1-3	4-7	8-14	15-31 22+
UCH	182 men	67	71	76	81*	—
	88 women	52	58	76	86	88*
St Giles	133 men	97	—	100	—	—
	76 women	92	93	97	98	100
JPH	295 men	78	79	85	91	95
	96 women	60	61	79	87	88
Radcliffe	59 men	92	93	100	—	—
	50 women	64	68	84	90	94
STH	255 men	90	93	95	98	99
	145 women	59	75	90	94	96

*Where percentages do not add up to 100%, data were not recorded or inaccurately recorded

Table 3 *Percentage of patients interviewed/reinterviewed*

Clinic	Gender of patients	Interviews				Total no. patients	Total no. interviews
		One	Two	Three	Four or more		
UCH	Men	91	8	—	—	182	200
	Women	100	—	—	—	88	88
St Giles	Men	69	19	10	2	133	192
	Women	70	26	4	—	76	102
JPH	Men	91	9	—	—	295	321
	Women	96	3	—	1	96	102
Radcliffe	Men	59	39	2	—	59	84
	Women	74	20	4	2	50	67
STH	Men	24	50	20	6	255	426
	Women	41	46	9	4	145	257

Table 4 *Total number of contacts named and contacts visited*

Clinic	Gender of contacts	Total no. of contacts		Total no. of visits made to contacts
		Named	Visited	
UCH	Male	212	—	—
	Female	186	1	1
	Not recorded	1	—	—
St Giles	Male	134	5	7
	Female	154	5	5
	Not recorded	21	—	—
JPH	Male	331	1	2
	Female	229	2	2
	Not recorded	4	—	—
Radcliffe	Male	110	1	1
	Female	98	8	8
	Not recorded	1	—	—
STH	Male	274	6	14
	Female	335	13	20
	Not recorded	2	—	—

The information recorded for such a located contact should include patient number, name of clinic, date of first attendance, and diagnosis.

The health worker takes the first step in this process at the first interview with a patient; assessment is made of the probability of bringing a contact

to examination, and what action the patient and/or health worker may initiate to achieve this. It was possible to deduce from the information recorded on the contact cards what assessment the health worker had made. The kinds of assessments made were categorised as follows:

- Contact clearly untraceable
- Contact believed to be already attending a clinic
- Patient to inform contact
- Patient probably unable to inform contact
- Health worker to inform contact
- Special contact report sent to DHSS/other clinic
- Other, including contact named by someone other than patient; patient to try and inform contact through a third person; contact named, but involvement in infection questioned.

Table 5 shows how health workers assessed the possible outcome of contact investigation, related to the gender of the contacts. Few special contact reports were sent, so this category has been omitted.

These findings illustrate the difference that the gender of the contact made in this assessment. A higher proportion of men were thought to have attended a clinic before the patient was interviewed, and a higher proportion of women were yet to be informed by the patient naming them. This finding is not difficult to explain, given the differences in symptoms between men and women. The assessment of 'untraceable' and that of 'probably unable to inform' was made in a higher proportion of cases where the contact was a woman. The low proportion of contacts who were identified for 'health worker to inform' was surprising.

THE OUTCOME OF CONTACT INVESTIGATION

It was possible to code the outcome of contact tracing in one of four ways:

1. Contact known to have been examined;
2. Contact believed to have been examined;
3. Contact informed, but outcome unknown;
4. Contact not known to have been examined.

In category 1, proof of attendance was required—that is the name of clinic, patient number, and date of attendance recorded on the contact card. Category 2 was for those cases where there was reason to believe that contacts had attended a clinic or doctor for examination either privately or abroad. Although definite proof of attendance was lacking, there was a considerable degree of certainty attached, although it was not possible for a health worker to verify the information. Category 3 were those cases in which there was evidence that the contact had been informed of the need for examination, but the outcome was either not known or too uncertain to meet the criteria. Contacts were assigned to cate-

Table 5 *Assessment of possible outcome of contact investigation, by gender of contact*

Initial assessment	UCH		St Giles		JPH		Radcliffe		STH	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Untraceable (%)	13	19	6	16	12	21	5	10	7	7
Believed attending (%)	40	15	37	19	29	15	47	16	49	16
Patient to inform (%)	37	52	32	45	43	48	29	54	26	50
Patient probably unable to inform (%)	5	8	10	12	6	8	2	6	4	13
Health worker to inform (%)	1	2	2	2	3	3	10	4	6	4
Other (%)	—	3	2	4	1	2	3	5	2	7
Unrecorded (%)	5	2	12	2	5	3	4	1	4	3

category 4 when there was no evidence to suggest that they had been informed or examined. It was not possible to assess how many of these contacts, or indeed those in category 3, actually and ultimately did attend for examination.

Table 6 shows the percentage and gender of contacts assigned to each outcome category. It can be seen from Table 6 that more than half of the male contacts, in all clinics, were known to have been

examined. The highest figure (64%) was for male contacts of patients attending the Radcliffe clinic. About half of the female contacts were known to have been examined; the highest figure of 58% was also obtained at the Radcliffe. Not surprisingly, more female than male contacts were *not* known to have been examined. This is true for all the clinics.

Table 7 compares the assessment made at first interview with the recorded outcome of contact

Table 6 *Outcome of contact tracing related to gender of contact*

Clinic	Gender of contact	Category				Total no. of contacts
		1. Contact known to have been examined (%)	2. Contact believed to have been examined (%)	3. Contact informed, outcome unknown (%)	4. Contact not known to have been examined (%)	
UCH	Male	53	4	4	39	212
	Female	50	—	2	48	186
	Not recorded	—	—	—	—	1
St Giles	Male	59	11	5	25	131
	Female	45	7	1	47	154
	Not recorded	24	—	5	71	21
JPH	Male	51	22	4	23	331
	Female	47	12	6	35	229
	Not recorded	—	—	—	—	4
Radcliffe	Male	64	8	4	24	110
	Female	58	—	11	35	97
	Not recorded	—	—	—	—	1
STH	Male	56	11	10	23	274
	Female	50	4	4	42	335
	Not recorded	—	—	—	—	2

Table 7 *Assessment compared to outcome*

Clinic	Outcome	Believed already attending	Patient to inform	Patient unable to inform	Health worker to inform	Untraceable	Total no. of contacts
UCH	Definitely examined	101	92	—	—	—	193
	Believed examined	9	1	—	2	—	12
	Not known examined	2	81	26	3	63	175
St Giles	Definitely examined	62	69	—	2	2	135
	Believed examined	15	8	—	—	—	23
	Not known examined	2	40	35	4	40	121
JPH	Definitely examined	94	152	4	14	1	265
	Believed examined	36	53	3	—	—	92
	Not known examined	1	49	30	2	90	172
Radcliffe	Definitely examined	64	48	—	9	—	121
	Believed examined	3	5	—	1	—	9
	Not known examined	1	31	8	5	17	62
STH	Definitely examined	152	134	2	16	—	304
	Believed examined	27	11	2	1	—	41
	Not known examined	9	94	50	13	42	208

investigation. The categories of assessment which were seldom used have been omitted from this comparison.

Health workers were able to establish in most cases a definite date and place of examination for a contact thought to be already attending a clinic or doctor. An outcome of 'definitely examined' was found for slightly more than half of those contacts in the category 'patient to inform'. Three of the 253 contacts assessed as untraceable were subsequently recorded as examined.

Table 8 shows whether contacts attended before, after, or on the same day as the patient naming them. Some records which were left incomplete were classified as 'no interval recorded'.

The sex difference is noticeable; with the exception of JPH, over half of the men named as contacts had attended *before* the patients naming them. More than half of the women attended *after* the patients naming them. The relative proportion of contacts attending on the same day as the patients naming them was similar for the four London clinics. The Radcliffe figures differ sharply, reflecting the fact that clinic sessions for men and women were held on alternate days.

Table 9 shows where contacts attended for medical examination. The 'other clinic' category includes clinics in and outside London, and in and outside the UK. Contacts included in the 'other/not applicable' category did not attend clinics but consulted GPs, private doctors, or other hospital doctors.

For all contacts known to have been examined, the majority attended the same clinic as the patient who named them. Fewer male contacts attending the same clinic as the patient naming them were recorded at JPH than in any of the other clinics. There is no explanation for this finding. The comparatively high percentage of male contacts of Radcliffe patients who were not treated in a clinic

Table 8 *Percentage of contacts who attended before, on same day, or after patient attended*

Clinic	Gender of contact	% of contacts attending			% for whom no interval recorded	Total no. of contacts
		Before	Same day	After		
UCH	Male	59	18	17	6	108
	Female	24	21	53	2	85
St Giles	Male	54	13	27	6	83
	Female	16	20	54	10	76
JPH	Male	24	10	34	32	210
	Female	12	13	50	25	121
Radcliffe	Male	54	4	30	12	77
	Female	19	4	74	3	57
STH	Male	52	15	20	13	172
	Female	16	18	56	10	175

Table 9 *Place of examination of contacts*

Clinic where patient attended	Place of examination of contacts	Male contacts		Female contacts	
		(%)	(no.)	(%)	(no.)
UCH	Same clinic	72		72	
	Other clinic	22		28	
	Other/not applicable	4*		—	
St Giles	Same clinic	79	120	74	94
	Other clinic	13		17	
	Other/not applicable	8		9	
JPH	Same clinic	53	86	66	78
	Other clinic	33		22	
	Other/not applicable	11		12	
Radcliffe	Same clinic	75	230	83	123
	Other clinic	5		12	
	Other/not applicable	20		5	
STH	Same clinic	70	79	67	58
	Other clinic	18		28	
	Other/not applicable	11*		4*	
			176		175

*In instances where percentages do not add up to 100%, data on place of examination were not recorded.

may reflect the fact that there is only one clinic in the locality although a proportion may have been examined at the local USAAF base. However this pattern was not found among the female contacts of Radcliffe patients.

Ninety-three per cent of patients interviewed during the study period had gonorrhoea, and Table 10 summarises the diagnoses of their contacts. All those contacts known or believed diagnosed are included in this table. The category 'diagnosis questioned' was used when the diagnosis was in doubt.

It can be seen that, with the exception of the Radcliffe, more female than male contacts had gonorrhoea. About 70% of male contacts had gonorrhoea and this figure was nearly 75% for females. Only a small percentage was found not to have any infection at all. Differences between males and females and between clinics may reflect differences in (1) clinic policies, (2) laboratory facilities, (3) health worker decisions about which sexual partners are involved in a given infection, or (4) they may indicate a real sex difference in infectivity, or any combination of these factors.

Conclusions

1. Many patients were willing and able to give reliable information to the health worker about contacts:

- (i) in those instances where contacts were believed to be already attending a clinic, the health workers were able to establish an outcome of 'known to have been examined' for at least

Table 10 *Diagnosis of contacts of patients with gonorrhoea*

Clinic	Gender of contact	Percentage known or believed to have			Percentage with diagnosis		No. of contacts known/believed diagnosed
		Same infection as patient	Different infection from patient	No infection	Questioned	Not recorded	
UCH	Male	75	6	12	6	1	121
	Female	88	6	5	1	—	82
St Giles	Male	71	3	8	16	2	84
	Female	81	4	3	12	—	67
JPH	Male	57	4	3	19	17	207
	Female	65	3	5	13	14	101
Radcliffe	Male	70	8	16	6	—	64
	Female	72	14	8	4	2	72
STH	Male	71	15	6	5	3	187
	Female	81	7	7	1	4	134

70% of contacts in all clinics and 94% in one.

- (ii) in those instances where the patient had agreed to inform the contact, between 50% and 60% of contacts were subsequently known to have been examined.

The implication of these findings is that most patients *do* understand the responsibility of informing sex partners about the risk of infection and do so. The indirect influence of the health worker on this transaction is impossible to determine. Only in those few cases where it is left to the health worker to inform a known contact at risk is it possible to determine if direct action is successful.

2. More men than women were interviewed by the health worker on the day that they first attended the clinic, as more men are diagnosed and treated on their first attendance.

3. Much of the health worker's time is spent in verifying the information given about contacts. Some health workers think that their time is wasted on 'unnecessary' clerical work, but the administrative aspect of the health worker's job is an integral part of it and is essential to measure effectiveness. The reinterview is an opportunity for the health worker to:

- (i) discover how effective the patient has been in locating and informing his contacts
- (ii) determine what further action may need to be taken by the patient or directly by the health worker. Great variation was found in clinic practice regarding reinterview and it is recommended that health workers routinely try to interview patients on at least one subsequent clinic visit as it is the most efficient way to determine outcome.

4. As with the patient population, differences were observed between men and women in the contact population.

- (i) At first interview, female contacts were more often assessed as untraceable than male contacts. Fewer women than men were thought

to be already attending a clinic. A higher proportion of women were to be informed by the patient naming them and the assessment of 'patient probably unable to inform' was made far more often when the contact was a women.

- (ii) Male contacts were more often known to have been examined and believed to have been examined than female contacts. More than half of the male contacts, in all clinics, were known to have been examined, compared with rather less than half of the female contacts.

- (iii) Most male contacts attended before or on the same day as the patient who named them. This highlights again the fact that males with urethral gonorrhoea are likely to have symptoms which trigger attendance, whereas women attend in response to having been told of a partner's symptoms or confirmed infection.

5. Two-thirds or more of those contacts who were examined attended the same clinic as the patient who named them. This finding should reassure health workers that it is possible to complete much contact investigation within one clinic.

6. At least 85% of those contacts who were examined were found to have a treatable condition. At least 67% had the same infection as the partner who named them. A slightly higher percentage of female contacts than male contacts (with the exception of the Radcliffe) were found to have the same infection as their partners.

The concept of success or effectiveness of contact tracing is complex in definition and measurement. It is not surprising that health workers have difficulty in determining the success of their efforts. Until now they have not been in a position to examine how they might work more effectively. It is suggested that the job satisfaction and status of health workers in special clinics depend on the acceptance of a

system that, using care in both definition and recording, gives a measurable standard of effectiveness.

The study described in these two papers was supported by the Medical Research Division and the Education and Training Division of the Health Education Council. We should like to thank the consultants and health workers in the five clinics for their co-operation and criticism; also Chris Hall of

Bromley Social Services for his help in preparing the tables and all those who helped collect and code the information.

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A survey of attitudes of patients in a department of genitourinary medicine

M. J. BALSDON, B. CHARLTON, M. CURRAN, AND H. S. K. SINGHA

From the Departments of Genitourinary Medicine and Community Medicine, Southampton University Hospitals, Southampton

SUMMARY A questionnaire concerning clinic organisation was completed by 415 patients attending a department of genitourinary medicine. Most (62.9%) patients preferred to be identified by number rather than by name, and about half (52.7%) did not like mixed waiting rooms. An appointment system as well as having an open clinic was liked by twice as many (44.9%) as those patients (23.6%) against such a system. More information about sexually transmitted diseases in the form of leaflets, etc. at the clinic was requested by most (79.1%) patients and a similar number (82.0%) felt the department itself should be more widely advertised.

Introduction

The Venereal Disease Service in Great Britain was established sixty years ago. Despite radical changes in social conditions and in sexual behaviour and attitudes since then methods of organisation and practice at clinics have changed little in many departments. It has been suggested that the modern clinic dealing with sexually transmitted diseases should be organised in a manner similar to other medical clinics (Catterall, 1973). A document on the planning and design of larger departments has been issued by the Department of Health and Social Security (1974), but no information has been published on the attitudes of the patients to the design and organisation of clinics of genitourinary medicine.

The present department of genitourinary medicine at Southampton is situated near a main hospital—the Royal South Hants. Patients are called by number, there is no appointment system, and there are separate waiting areas for men and women. A small amount of reading matter on sexually transmitted diseases, mainly pamphlets, is available in the waiting areas.

It was decided to assess the patients' attitudes towards these aspects of the clinic organisation and routine to help in making decisions about a new department planned as part of the new Royal South Hants Hospital.

Materials and methods

A questionnaire was drawn up and tested in a pilot survey. It was handed out by two medical students (BC and MC) who also collected the completed forms. This study formed part of the students' fourth year research project (Normand and Cantrell, 1976). Patients filled in the questionnaire while in the waiting room before seeing the doctor. Patients replied to the questions by ticking the appropriate boxes. One question had a space so that the patient could suggest places in which the clinic could be advertised, and there was also a space for 'other comments'. All clinic sessions were covered during the three-week study period.

Results

Four hundred and fifteen questionnaires were completed by patients (262 by men and 153 by women). Seventy-one per cent of the patients were under 30 years of age. The replies to the first four questions are summarised in Table 1. Replies from men and women, and old and new patients, did not differ significantly so these have been amalgamated. More reading matter on sexually transmitted diseases at the clinic was requested by 79.1% of the patients. A similar number (82.0%) felt that the clinic should be advertised more. Sites mentioned by patients for advertising the clinic are shown in Table 2.

Address for reprints: Dr M. J. Balsdon, Royal South Hants Hospital, Department of Genitourinary Medicine, Buller Street, Southampton SO2 0NH

Received for publication 28 November 1977

Table 1 *Replies to questionnaire*

Question	Answer %		
	For	Don't mind	Against
Do you like the present system of being called by number?	62.9	33.2	3.9
Would you prefer to be called by name?	12.7	34.4	52.8
Would you like an appointment system at the clinic as well as the present arrangements?	44.9	31.4	23.6
Would you like husbands and wives or boyfriends and girlfriends sitting together in the waiting room?	22.8	24.4	52.7

Table 2 *Sites mentioned for advertising*

Sites	Males	Females
Local newspapers and magazines	28.8	23.6
Public places*	22.3	23.0
Bars, clubs, and cinemas	10.2	4.4
Television and radio	11.3	3.7
Public lavatories	8.0	6.2
Colleges, schools, etc.	6.6	9.9
Doctors' surgeries, clinics, waiting rooms	5.5	16.8
Others	7.3	12.4
Total (no. of times mentioned)	100.0% (274)	100.0% (161)

*Post offices, railway stations, etc.

Discussion

The fact that most patients in Southampton preferred to be identified by a number at the clinic was an interesting finding in view of the dissatisfaction expressed by some patients before the survey. The open nature of the waiting areas (they are really main corridors in continuous use by staff and patients) and the lack of adequate soundproofing in the Portakabin structure may be factors. Perhaps there should be more emphasis on confidentiality rather than anonymity. The percentage of patients who preferred the present system of numbers compared with the use of names for identification was approximately equal, confirming the strength of this finding.

Many patients would welcome an appointment system in addition to the present 'open' clinic arrangements. An appointment system for patients with difficult or protracted problems would ensure 'same doctor' management which was a point made by several patients in the questionnaire. A suitably organised appointment clinic would also ensure that medical students would see a constant flow of

patients rather than erratic patient attendance as at the present open clinic.

Waiting rooms for men and women together were disliked. Although Southampton is a large city some patients stated that they might feel embarrassed, particularly if their contacts attended at the same time. This finding may again be related to the physical nature of the waiting areas. It is interesting to note that the smallest clinics in the specialty often have mixed waiting rooms and complaints from patients seem to be few. Perhaps the best solution would be to have a 'family room' for partners as well as 'singles' waiting rooms. Some clinics already have this arrangement.

Reading material on sexually transmitted disease is commonly requested by a patient. This request may be related to unwillingness to ask questions owing to embarrassment or because there is no time for adequate explanation in a busy clinic. Since the survey there has been a comprehensive stock of pamphlets in the waiting area and this has been much appreciated by patients.

There was general agreement that the clinic should be advertised more widely. The sites mentioned by patients for advertising may to some extent reflect individual life styles, but a quarter of all patients who mentioned sites quoted local newspapers and magazines. The female patients mentioned surgeries, clinics, and waiting rooms often enough to make these also worthy of consideration. In view of the interest shown in advertising in local newspapers we are considering having a suitably worded advertisement.

It was hoped that the replies to this questionnaire would give some indication of the patients' feelings on aspects of clinic organisation and this has been achieved.

We thank the staff and patients of the department for their help and co-operation and Professor W. E. Waters and Dr D. J. B. Barker of the Department of Community Medicine, Southampton University, for their advice.

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Which patients with sexually transmitted diseases default?

Report of a survey in one clinic

S. PERFREMENT AND K. OVERFIELD

From the University of Sussex and the Department of Genital Medicine, St Bartholomew's Hospital, London

Introduction

Little is known about the motivation of patients who attend clinics for the treatment of sexually transmitted disease (STD). Thin and Smith (1976) reported the source of attendance (self-referral, contact action, referral by a doctor, or other source) for heterosexual and homosexual men attending an STD clinic. However, no account was taken of the attitudes of these men towards STD or their motivations to continue to attend when advised to do so.

It was therefore decided that patients attending an STD clinic should be studied to try to identify groups (either by demographic factors or by the patients' attitudes) who were likely to complete their course of treatment and follow-up.

Method

Two pilot studies were first carried out; then 100 patients attending between January and March 1976 were interviewed. A questionnaire with standardised, pre-set questions was administered by one of us. All those interviewed were attending the Department of Genital Medicine, St Bartholomew's Hospital, London for the first time, although some of the patients had previously attended another STD clinic. The interview took seven minutes and was at the end of the patient's visit. The coded responses were then punched on 80-column cards and a FORTRAN-based computer program was used to analyse them.

THE QUESTIONNAIRE

This sought the following information:

1. Demographic data—such as age, sex, marital state, and social class.

2. How the patient found out about the clinic.
3. The patients' concepts of their attitudes to STD and clinics before attending, and any changes they thought had taken place during their visit.
4. Reactions to the clinic and its staff formed during their first visit, and any improvements the patients thought could be made.
5. Details of attendance and diagnosis, obtained one month after the interview. A 'defaulter' was taken to be any person who failed to return during that month when she or he had been asked to do so.

AN 'OCCUPATION/CLASS' SCALE

As the Registrar General's classification was considered to be inappropriate for the purpose of determining educational level and, as education is important in the formation of attitudes, a special scale—linked to educational qualifications—was devised. This comprised the seven following categories:

1. Higher professional (HP)—for example, chartered accountant, lecturer, company director, or architect.
2. Lower professional (LP)—for example, nurse, librarian, journalist, or insurance broker.
3. Clerical—for example, bank clerk, insurance representative, or telephonist.
4. Manual—for example, machinist, driver, postman, or market worker.
5. Unemployed.
6. Student.
7. Housewife (any married woman who had an occupation was classified under that occupation).

THE DIAGNOSTIC CATEGORIES

These were as follows:

1. Syphilis or gonorrhoea.
2. Minor STDs including non-specific urethritis (NSU), non-specific genital infection (NSGI),

Address for reprints: K. Overfield, 9 Clifton Road, Brighton, Sussex BN1 3HP

Received for publication 14 June 1977

candidosis, trichomoniasis, balanitis of undetermined origin, scabies, pediculosis, genital warts, and genital herpes.

3. No STD, but treated for other conditions.
4. No disease found.

Comparison of our sample with all new cases attending the clinic during the same quarter of the year showed that it was representative, except that there were fewer patients with gonorrhoea and syphilis in our sample.

Results

DEMOGRAPHIC DATA

Only data of special relevance are given.

Age

Over half (53%) of patients were in the 20–29 year age group, Table 1. The sex ratio was such that, for approximately every two women attending, there were three men (male : female ratio 3:2).

Marital state

Single—55%, married—34%, cohabiting—2%, separated—4%, divorced—5%.

Occupation/class

HP—11%, LP—42%, clerical—22%, manual—14%, unemployed—1%, student—8%, housewife—2%.

Table 2 shows that only three patients had syphilis or gonorrhoea; most patients who attended had less serious STDs.

PATIENTS' ATTITUDES BEFORE ATTENDING AND ATTENDANCE PATTERN

Table 3 shows that most (79%) interviewees were attending an STD clinic for the first time. Just over one-fifth (21%) had previously attended elsewhere. Nevertheless the patients attending for the first time were likely to be less 'positive' (as defined in Table 3) in their attitudes to coming to a clinic than those who had been to other clinics. Those patients attending a clinic for the first time were also much more likely to have expected the visit to be worrying or embarrassing.

Table 4 shows the relationship between having attended another clinic and the patients' eventual attendance pattern at the Department of Genital Medicine. Although approximately two in three

patients did return as asked, patients who had previously been to another clinic were more likely to default (9/20 or 45%) than those who had never been to another clinic (23/73 or 31.5%).

FINDING OUT ABOUT THE CLINIC

Most (62%) interviewees had found out about the clinic from their partners, friends, or doctors (Table 5).

Table 2 *Diagnosis and gender of patients attending the clinic*

Gender	Syphilis and gonorrhoea	Minor STDs	No STD*	No disease present	Total
Female	2	33	2	1	38
Male	1	54	4	3	62
Total	3	87	6	4	100

*But treated for other conditions

Table 3 *Prior attendance at another clinic and patients' attitudes before attending Barts*

Attitude	Patients who had not attended before	Patients who had attended another clinic	Total
Positive*	24	10	34
Neutral†	45	9	54
Negative‡	8	2	10
Incomplete data§	2	0	2
Total	79	21	100

*Positive: the patient expected the staff to be friendly and the visit not to present him/her with any problems

†Neutral: the patient had neither good nor bad expectations

‡Negative: the patient expected the staff to be unpleasant and for it to be a worrying and embarrassing experience

§Incomplete data: due to interviewing/programming error

Table 4 *Prior attendance at another clinic and eventual attendance pattern at Barts*

Attendance	Patients who had not attended before	Patients who had attended another clinic	Total
Returned as asked	50	11	61
Did not return, defaulted	23	9	32
NA/NR*	6	1	7
Total	79	21	100

*Not applicable no response

Table 1 *Age and gender of patients attending the clinic*

Gender	Age (years)					Total
	16–19	20–29	30–39	40–49	50–62	
Female	3	25	7	2	1	38
Male	5	28	14	12	3	62
Total	8	53	21	14	4	100

Table 5 *How the patients found out about the clinic*

Contacted, referred, told by sexual partner or by GP	44
Recommended by friend	18
Notice or poster	16
Already knew of clinic from visits to other departments	12
From directory or guide	10
Total	100

CHANGES IN ATTITUDE TOWARDS STD DURING PATIENTS' VISIT

Overall 57% of patients did not think their attitudes to STD had changed since their visit to the Department of Genital Medicine. The 43% who did report changes in attitude are shown in Table 6a. If all patients' final attitudes are analysed, the result is as shown in Table 6b.

PATIENTS' REACTIONS TO THE CLINIC

Table 7 shows that, at the end of the first visit, most patients felt the staff were friendly, reassuring, and capable—this was especially the case for the female patients.

Only 39 patients suggested improvements. The one most often wished for was that there should be greater privacy initially in the waiting area; this was given by nine respondents.

Fifty-eight of the 59 patients who had asked the doctor questions during their visits considered that

these had been satisfactorily answered. In response to another question during the interview, 74 patients thought it would be a good idea if written information about STD were provided in the clinic, while only 19 were against this idea.

SUBSEQUENT ATTENDANCE

Twenty patients said that they would find it difficult to carry out the instructions given to them by the doctor—such as abstaining from sexual intercourse and from alcohol and returning to the clinic. Nine said further attendance would be inconvenient, six said it would be awkward to get time off work, and five that it would be difficult for personal reasons. Further analysis showed, nevertheless, that an *inverse* relationship existed between patients' concept of possible problems, and their subsequent attendance—in other words, those who felt it would be difficult to follow instructions were also *more* likely to be regular attenders.

DEFAULTERS

The attendance records showed that 32 patients defaulted within the first month. Certain factors seemed to be more important than others. These were:

1. The default rate was markedly higher among those who had been to another clinic before (see Table 4).
2. The proportion of defaulters was also higher among those who, after the visit, changed from being embarrassed about STD to considering STD as an ordinary illness—that is, 13 of 24 cases, or 54.2% (see Table 6a).
3. Those who had had 'positive' attitudes towards coming to a clinic before they visited it were more likely to complete their treatment (see Table 3).
4. Five (38.5%) of the 13 female patients diagnosed as having candidosis subsequently defaulted.
5. In the LP group (42% of the sample) 15 of 41, or 36.6% (one case incomplete data) defaulted; this is slightly higher than the overall default rate.

Table 6a Patients' reported change (if any) in attitudes to STD since attending Barts, and attendance pattern

Barts attendance	Final reported attitude				Total
	'Ordinary illness'*	Now embarrassed†	Don't know‡	NA/NR§	
Returned as asked	11	5	1	5	22
Did not return; 'defaulted'	13	2	0	3	18
NA/NR§	2	0	0	1	3
Total	26	7	1	9	43

*Changed from not knowing or feeling embarrassed to regarding STD as an ordinary illness

†Changed from not knowing or regarding STD as an ordinary illness to feeling embarrassed

‡Changed from feeling embarrassed to not knowing how he or she felt

§Not applicable/no response

Table 6b All patients' final reported attitudes to STD

STD seen as 'ordinary illness'	54
Patient worried or embarrassed by subject	29
Don't know	7
Not applicable/no response	10
Total	100

Table 7 Attitudes towards the clinic staff at the end of the first visit by gender of patient

Gender	Positive*	Neutral†	Negative‡	NA/NR§	Total
Female	30	5	1	2	38
Male	39	16	4	3	62
Total	69	21	5	5	100

*Positive: the patient found the staff reassuring, capable, and friendly

†Neutral: the patient felt indifferent towards the staff

‡Negative: the patient did not find the staff reassuring, capable, or friendly

§Not applicable/no response

Discussion

In any discussion of the default rate, it must be remembered that our sample was small. However, the 32 patients who defaulted nevertheless represent nearly one-third of the sample. Demographic factors—such as, age and gender—generally had little influence on patients' attitudes and attendance patterns, but some groups had more defaulters than others.

The first group were those patients who had previously been to another clinic, or those who, after visiting this clinic, considered STD as an ordinary illness. It may be that they regarded their problem too lightly and did not realise the potential seriousness of STD. Perhaps the medical staff put insufficient emphasis on the importance of coming back (even when symptomless) and on completing the course of treatment and/or follow-up, and did not give reasons for this clearly enough.

In the second group were the female patients with candidosis (13% of the clinic's clientele) who throughout tended to show more negative or reserved attitudes. One possible explanation is that candidosis tends to be perceived as 'only thrush' and the fact that it may be an STD is forgotten. Perhaps patients are not made sufficiently aware of the implications of candidosis in this respect.

Thirdly, the higher default rate among the LP group suggests that the approach of the clinic is not right for these people, or vice versa.

In view of the type of person likely to default, and the fact that 74% of the patients wanted more information, we suggest that such information should be provided. This should be aimed at the 18-30 year age group, who comprise 60% of patients, and at the HP, LP, and clerical groups, who together make up 75% of the sample. Ideally, this information should be available during the visit so that the patient has time to ask questions about it before he

or she leaves. The cards or pamphlets should be given to the patient, not simply left around to be picked up as many people are unwilling to do this.

Conclusions

Comparison with all new cases attending this clinic in the first quarter of 1976 indicated that our sample was representative. We would, however, have reservations about applying our findings to other clinics. The social characteristics of the patients attending this clinic are due to its geographical position in the City of London.

The results of our survey indicate that the clinic does influence attitudes (which may affect the attendance rates), although it would seem that these are very likely to be linked also to external factors, such as social class or previous attendance at an STD clinic. There is a need for more research in this area.

We thank Dr R. N. Thin for permission to carry out research in the DGM, and the staff for their kind co-operation.

Reference

- Thin, R. N., and Smith, D. M. (1976). Some characteristics of homosexual men. *British Journal of Venereal Diseases*, 52, 161-164.

Correspondence

TO THE EDITOR, *British Journal of Venereal Diseases*

Sir,

Tetracycline treatment for non-specific urethritis

Mr Simopoulos (1977), having drawn attention to the divergent views held regarding the value of tetracycline treatment in non-specific urethritis (NSU), states that these incompatible views suggest that the whole system of investigation devised to validate these views is inappropriate. He suggests criteria which he believes would allow effective evaluation of treatment. These criteria have been in use for many years in this clinic. However the long follow-up was shown to be unnecessary in a study (Fowler, 1970) in which the follow-up was not the one year Mr Simopoulos finds so admirable, but three years, and the long-term findings proved of no help in assessing treatment.

Most of the criteria suggested by Mr Simopoulos were necessary in the past when the aetiology and the natural course of NSU were obscure and we needed treatment for the condition as a whole. It is questionable whether such an approach is still valid. It may be more helpful to try to distinguish the cases in which there is a marker—such as *Chlamydia trachomatis*, *Trichomonas vaginalis*, or the herpes simplex virus—or some other identifiable feature. In this way we may explain the apparently irreconcilable findings that led Weston (1965) to suggest that allergy may have an important aetiological role, Grimble and Amarasuriya (1975) to postulate mixed infections, and Evans (1977) to implicate psychological factors.

References

- Evans, B. A. (1977). The role of tetracyclines in the treatment of non-specific urethritis. *British Journal of Venereal Diseases*, 53, 40-43.
Fowler, W. (1970). Studies in non-gonococcal urethritis therapy. The long-term value of tetracycline. *British Journal of Venereal Diseases*, 46, 464-468.
Grimble, A. S., and Amarasuriya, K. L. (1975). Non-specific urethritis and the tetracyclines. *British Journal of Venereal Diseases*, 51, 198-205.
Simopoulos, J. Ch. (1977). Tetracycline treatment for non-specific urethritis. *British Journal of Venereal Diseases*, 53, 230-232.

Weston, T. E. T. (1965). An allergic basis for non-specific urethritis. *British Journal of Venereal Diseases*, 41, 107-116.

Yours faithfully,

W. Fowler

Department of Venereal Diseases,
The General Hospital,
Steelhouse Lane,
Birmingham B4 6NH

TO THE EDITOR, *British Journal of Venereal Diseases*

Sir,

In his paper (Simopoulos, 1977) Mr Simopoulos questions our arithmetic concerning the number of defaulters in the first week of surveillance. Perusal of the fourth columns of Tables II and III of our paper (Masterton and Schofield, 1972) will confirm that our statements concerning last attendances before subsequent default are correct:

Day	Subsequent default	
	(Table II)	(Table III)
2	16	17
4/5	3	12
8	4	8
Totals	23	37

We fail to see how these figures could be taken to 'incline the reader to caution' in accepting our results.

Mr Simopoulos also chides us for not having used a placebo in the trial. We do not believe it ethical to deny treatment to patients who have a potentially sexually transmissible disease with known complications. We always use the results of our previous drug trials, in all of which the same protocol was used, as controls for our current drug trial. The results are therefore comparable.

References

- Masterton, G., and Schofield, C. B. S. (1972). Doxycycline HCl (Vibramycin) as a single dose oral treatment of gonococcal and nonspecific urethritis in men. *British Journal of Venereal Diseases*, 48, 121-125.

Simopoulos, J. Ch. (1977). Tetracycline treatment for non-specific urethritis. *British Journal of Venereal Diseases*, 53, 230-232.

Yours faithfully,

G. Masterton
C. B. S. Schofield

Newcastle General Hospital,
Westgate Road,
Newcastle upon Tyne, NE4 6BE

TO THE EDITOR, *British Journal of Venereal Diseases*

Sir,

The paper by Simopoulos (1977) deserves comment. We agree that there are flaws, some of them serious, in studies which have been carried out to determine the effectiveness of tetracyclines in the treatment of non-specific urethritis. However, the author concludes among other points that studies should be placebo-controlled and should have a prolonged follow-up period. There is no doubt that tetracycline treatment is effective, at least over the short term. Placebo-controlled studies in the past (Csonka, 1965), including those that have taken into account the microbiological aspects (Holmes *et al.*, 1967; Prentice *et al.*, 1976), have shown this to be so. It is therefore clear that to deny treatment to patients in a placebo-controlled study would now raise severe ethical problems. Furthermore, the sexual activity of patients cannot be accurately monitored over a long period unless there are most unusual circumstances (for example, aircraft carriers, Antarctica). There is, therefore, no question that it is difficult, indeed impossible, to judge the results of treatment over a long time, since reinfections are not only prone to interfere (Lassus *et al.*, 1971), but do. It seems to us that these factors are vital in the interpretation of any antibiotic study. We do not believe that the author's criticism of other workers' studies helps to reveal anything that is not already appreciated, and we feel that in his comments about placebos and follow-up he shows a remarkable lack of appreciation of the problems.

References

- Csonka, G. W. (1965). Non-gonococcal urethritis. *British Journal of Venereal Diseases*, 41, 1-8.
- Holmes, K. K., Johnson, D. W., and Floyd, T. M. (1967). Studies of venereal disease. III. Double-blind comparison of tetracycline hydrochloride and placebo in treatment of nongonococcal urethritis. *Journal of the American Medical Association*, 202, 474-476.
- Lassus, A., Perko, R. L., Stubb, S., Mattila, R., and Jansson, E. (1971). Doxycycline treatment of nongonococcal urethritis with special reference to T-strain mycoplasmas. *British Journal of Venereal Diseases*, 47, 126-130.
- Prentice, M. J., Taylor-Robinson, D., and Csonka, G. W. (1976). Non-specific urethritis. A placebo-controlled trial of minocycline in conjunction with laboratory investigations. *British Journal of Venereal Diseases*, 52, 269-275.
- Simopoulos, J. Ch. (1977). Tetracycline treatment for non-specific urethritis. *British Journal of Venereal Diseases*, 53, 230-232.

Yours faithfully,

D. Taylor-Robinson,
M. J. Warrell,
G. W. Csonka

TO THE EDITOR, *British Journal of Venereal Diseases*

Sir,

Anal canal versus rectal wall sampling

In Dr Deheragoda's paper (Deheragoda, 1977) he reached the conclusion that rectal wall sampling should be confined to first visits and to patients with symptoms; but he does not seem to be justified by the results. Such information as he gives regarding male and female patients suggests that each year his department will miss up to 20 cases of gonococcal proctitis. Imitation of his proposals nationally and internationally, at a time when selective media and productive sampling of the rectum and pharynx are improving the control of gonorrhoea, would appear to be retrograde. Now that β -lactamase producers threaten serious problems it would not seem to be in the best interests of public health to miss cases of rectal infection.

Dr Deheragoda's recommendation engenders in me a sense of *déjà vu*. Many of us have spent much of our lives trying to bring doctors to a recognition that vaginal sampling is not the best way to diagnose gonococcal endocervicitis. It was said of high vaginal samples what is now said of anal canal samples—they are reliable, easy to perform, and not distressing to the patient.

I hope most sincerely that the department of genitourinary medicine at Charing

Cross Hospital will dissociate itself from these ideas and continue to be one of the United Kingdom's leading departments.

Reference

- Deheragoda, P. (1977). Diagnosis of rectal gonorrhoea by blind anorectal swabs compared with direct vision swabs taken via a proctoscope. *British Journal of Venereal Diseases*, 53, 311-313.

Yours faithfully,

R. S. Morton

TO THE EDITOR, *British Journal of Venereal Diseases*

Hepatitis B antigen and antibody in a male homosexual population

Sir,

Coleman *et al.* (1977) are to be congratulated on their detailed study of the incidence of hepatitis B antigen and antibody in the serum of 600 male homosexual patients, but it is difficult to accept their conclusion that there was little correlation between these indices of infection by the hepatitis B virus and a previous history of hepatitis, jaundice, or liver disease. The figures which they give can be compounded into the following table, which demonstrates a very strong correlation indeed between serological evidence of hepatitis B infection and a positive clinical history:

	History of jaundice, hepatitis, or liver disease		Total
	No history		
HBs Ag or IOEP-detectable			
HBs Ab	10	54	64
Neither	18	518	536
Total	28	572	600

$$\chi^2 = 19.34 \quad p < 0.0005$$

Furthermore, the authors state that the sera of 85 patients negative for HBs Ag and HBs Ab by routine methods were tested for antibody by radioimmunoassay (RIA) and three of them were found to have both antibody and a history of jaundice or hepatitis. If this proportion is representative of the whole series, it follows that among the 536 patients who

were negative for HBs Ag and immunosmoelctrophoresis—detectable HBs Ab there may well have been as many as 19 in whom the presence of RIA detectable HBs Ab coincided with a history of jaundice or hepatitis.

These 19, together with the 10 patients with a positive history and positive routine tests are sufficient to account for all the 28 patients in the series with a history of jaundice or hepatitis, so that it is likely that serological evidence of hepatitis B infection would have been found in every patient with a positive clinical history if the whole series had been tested for antibody by RIA. Finally, if the results of RIA testing of part of the series are extrapolated to the whole series it may be calculated that 42% of these men, almost all of whom had in all probability been exposed to the virus, probably had serological evidence of infection, and 11% of these had had a clinically apparent infection. This shows the extent to which the virus is dangerous, at any rate when acquired by homosexual means, and this to me is the main interest of this informative series.

Reference

- Coleman, J. C., Waugh, M., and Dayton, R. (1977). Hepatitis B antigen and antibody in a male homosexual population. *British Journal of Venereal Diseases*, 53, 132-134.

Yours faithfully,

P. H. Renton

National Blood Transfusion Service,
Roby Street,
Manchester M1 3BP

Notice

MSSVD Student Prize

The paper that won the MSSVD Student Prize competition in 1977 appears on page 160. This was the first year of this competition. The judges had great difficulty in choosing one of the seven entries, but used the following criteria:

1. The observations on which the report was based, appeared to have been made by the entrant alone.
2. The methods used to make the observations were of a high standard.
3. The report was clearly and concisely written.

It is hoped that by publishing these criteria future entrants will be helped.

Book reviews

Clinics in Rheumatic Diseases, Volume 3, No. 2. Immunogenetics and Rheumatic Disease. Edited by D. S. B. Inglis (Guest Editor: D. Brewerton), 1977. 381 pp, 42 tables, 12 figs. Saunders, Eastbourne (Price £8.25).

The discovery that some human leucocyte antigens (HLA) act as genetic markers for a number of rheumatic disorders has been described as perhaps the most significant advance in medicine during the last 25 years. Further study has shown that a wide range of diseases spanning almost the whole of medicine has been found to be involved in varying degrees with the HLA system. Despite the fact that knowledge of the association between immunogenetics and rheumatic disease is only five years old, information is accumulating rapidly and as the editor of this book (himself one of the team which made the first observations associating ankylosing spondylitis with HLA B27) states in his forward—'leading authorities have asked to review existing knowledge of the subject and to dwell on the many current problems and uncertainties'. This they have done in a widely ranging, detailed, and readable series of articles which manage to be as up to date as is possible in a book.

Excellent accounts are given of the major histocompatibility complex in man, immune responsiveness, and the relationship between rheumatic disease and the major histocompatibility complex. Although these sections will include much that is new to the average clinician they repay careful study.

Perhaps of most interest to the venereologist will be those sections dealing with ankylosing spondylitis, uveitis, Reiter's disease, and psoriatic arthropathy. In the section on ankylosing spondylitis intriguing information is given about the surprising findings obtained using HLA B27 detection as an 'epidemiological tool' in asymptomatic randomly selected populations. These preliminary findings showed a 20-30% prevalence of sacroiliitis and/or spondylitis which was equally apparent in both sexes. Previous studies had suggested prevalence figures of only 1-2% with a heavy male preponderance. A good account of the potential mechanisms of the association of B27 and spondylitis is given.

The section dealing with reactive arthritis, Reiter's disease, and psoriatic

arthropathy attempts to delineate the overlapping relationships between these conditions and reviews much interesting recent work. The distinction between 'reactive arthritis' and Reiter's disease is one which the experienced venereologist will find hard to understand (a point the authors accept). Study of the HLA systems and their association with these disorders has so far further established their inter-relationship but has done little to clarify it.

The problem of anterior uveitis, its clinical associations, epidemiology, immunology and investigation are well reviewed and there is a particularly useful bibliography.

In short, a valuable and readable book although perhaps rather expensive.

J. K. Oates

Gonorrhoea. Volume 9, Major Problems in Dermatology Series. R. S. Morton (Series Editor: A. Rook), 1977. Pp. 292, 24 tables, 56 figs. Saunders, Eastbourne (£12.00).

This beautifully produced monograph is the first comprehensive work on gonorrhoea to appear for many years. It thus bids to become a definitive reference on the subject, and in this role there can be no doubt of its success. It has been compiled with great thoroughness and accuracy, and the practical skill and long experience of the author are at all times apparent in the text. The historical chapter is the most detailed I have read, and provides a salutary reminder of the simplicity and potential effectiveness of treatment today. Laboratory aspects are condensed with expertise into a synopsis which embraces all, or almost all, important research work, most of which has appeared relatively recently. Unfortunately, local immunity receives only a single reference and one five-line paragraph (p. 52), which reflects neither the importance of the subject nor the amount of published work. Detailed histopathological descriptions appear in the chapters on gonorrhoea in both men and women, but without any attribution to the source. If it is Harkness, then the origin of much of his information is open to criticism on the grounds that it was not obtained from normal healthy young adults. The omission of an electron-micrograph of the gonococcus *in vivo* is unfortunate in the light of recent demon-

stration of morphological variation between this and the laboratory form by Novotny and others.

A few minor points also merit comment in an ambitious volume such as this. Perimetritis is surely more common in gonorrhoea than in NSU, rather than the reverse (p. 77). The description of the formation of cervical erosion (p. 106) is not in accord with currently accepted observations that stratified squamous epithelium is not actually replaced by columnar epithelium, but rather that the latter is exposed by hyperplasia and ectropion. In the chapter on prepubertal gonorrhoea, ophthalmia is illustrated by three pictures of chlamydial infection, two of which are colpophotographs, without real justification. Many would disagree that the urine test is normal within 72 hours of treatment of gonorrhoea in men (p. 149). The association between semen swallowing and hepatitis B antigen in the blood was subsequently shown to be false by the group who originally proposed it (p. 159). Transfer of infection by ano-rectal contact defies my imagination (also p. 159).

Dr Morton is again at his best in the chapter on control of gonorrhoea: the section on aims and methods is a masterpiece of condensation that could serve to guide everyone concerned with contact tracing. Both he and his contact tracing service are to be congratulated on examining no less than 78% of female contacts sought in 1976 (p. 247), a figure only dreamed of in London. This, and the monograph on gonorrhoea, bear witness to a distinguished career.

B. Evans

Nongonococcal Urethritis and Related Infections. Edited by D. Hobson and K. K. Holmes, 1977. Pp. 391. American Society for Microbiology, Washington DC (\$14.00).

Although it is not clear from the title, this is a report of the proceedings of a symposium held in the USA in April 1976. The first feature that strikes the reader is the large number of contributors and participants from the USA and Europe, and the variety of disciplines represented including genitourinary physicians, ophthalmologists, epidemiologists, pediatricians, veterinarians, and micro-biologists.

Some of these groups rarely, if ever, meet and to gather so many together is a remarkable achievement. This large, broad-based gathering of specialists also reflects the widespread interest in *Chlamydia* that has developed in the last few years.

Most of the contributions are of a high standard. At the end of each section is a useful summary of the original contributions and discussion. The subjects covered included the role of *Chlamydia trachomatis* and, less thoroughly explored, of *Ureaplasma urealyticum* in the aetiology of nongonococcal urethritis, and the diagnosis and treatment of this condition. Related genital infections, including epididymitis, prostatitis and pelvic infection, are also considered. A useful section to laboratory workers concerns the biology of *Chlamydia* and includes interesting studies of inoculation of experimental animals. Of more value to clinicians is a report from the Institute of Ophthalmology in London concerning antibiotic sensitivity. Rifampicin showed the greatest degree of activity against *C. trachomatis* sero type D, when tested in a cell culture system. Oxytetracycline, doxycycline, and aminocycline were next followed by erythromycin, spiramycin, and sulphamethoxazole. In another paper from Liverpool, it is shown that the effect of penicillin is complex, behaving as a chlamydiostatic rather than a chlamydiacidal agent. The laboratory methods for the diagnosis of *C. trachomatis* infections are considered in some detail and the final section is devoted to ureaplasmas.

One important contribution from Chicago concerns findings in neonates with *C. trachomatis*-positive conjunctivitis of *Chlamydia* in nasopharyngeal secretions. A small series is also reported in which a distinctive form of pneumonia was found. It is suggested that *C. trachomatis* infection commonly involves the lower respiratory tract. This form of pneumonia responded satisfactorily to treatment and apparently some cases recovered without any treatment. This is an important development in our knowledge of the clinical effects of *Chlamydia*.

A short review cannot do justice to this important book which contains 47 papers. It should be available to all those interested in the problems presented by nongonococcal urethritis, related infections, and *C. trachomatis*.

R. N. Thin

The Surveillance and Control of Sexually Transmitted Diseases. Report of a sym-

posium held 21-25 September 1976 in Vienna. 1977. Pp. 80. WHO, Copenhagen (price not quoted).

This report provides a useful summary of features of the British method of combating sexually transmitted diseases (STDs) and as such may be regarded as superfluous on the bookshelf of the genitourinary physician in the United Kingdom.

The importance of screening by general practitioners is emphasised, but the possession of the necessary equipment (for example darkfield microscope) is hardly likely to endear the process to many non-specialists, and the use of general screening campaigns for gonorrhoea is of dubious value in areas in which adequate STD clinics already exist or where there is a low level of STD in the community.

The authors come down heavily on the side of 'epidemiological treatment'—a term they consider 'less imprecise' (not unlike the diagnoses thus made) than 'rapid simultaneous treatment'. This is advocated for patients with an STD but not if they have non-specific urethritis (NSU), in which case, as some conference members said, syphilis or gonorrhoea might be masked. This is an interesting exception since most clinics are unable to diagnose non-specific genital infection in female partners of men with NSU due to lack of rapid cultural facilities for *Chlamydia* and *Mycoplasma*. The question of contact tracing in partners of those patients so treated 'simultaneously' is carefully avoided.

The report contains two useful, concise appendices on screening tests for STD and contact tracing. Page 25 was blank in the volume provided for review. The book is available on demand from the WHO Regional Office for Europe, Copenhagen.

P. D. Simmons

Gonorrhoea: Epidemiology and Pathogenesis. Proceedings of Symposium No. 2 held by the Federation of European Microbiological Societies on 15 and 16 November 1976. Edited by F. A. Skinner, P. D. Walker, and H. Smith, 1977. Pp. 255, 27 tables, 91 figs. Academic Press. London (£11.00)

This book records the proceedings of a symposium organised by the Society for Applied Bacteriology and the Society for General Microbiology on behalf of the Federation of European Microbiological Societies.

The subject is gonorrhoea. The research topics covered are wide; they include

the epidemiology and pathology of gonococcal infections, the problems of immunology, phagocytosis, and the attachment of gonococci to mucous surfaces. There is also a review of the treatment of gonorrhoea with antibiotics and the resistance of gonococci to them. These are all provided by experts in this field from Europe and America. All the papers are eminently readable and are enhanced by the summaries at the end of each one.

Gonorrhoea poses many problems and research has been launched in many directions. Although the answers to most questions have not been obtained, these papers record the direction of the investigations now in progress.

The symposium begins with a review of recent epidemiological studies in Sweden and draws attention to the importance of asymptomatic infection in male patients as well as in the female. The authors emphasise the increasing incidence of gonorrhoea in the pharynx and rectum and the recrudescence of gonococcal septicaemia in the last few years.

Diagnostic methods of culture and serology are then discussed. In recent times there has been an increasing interest in the use of serological methods in the detection of gonorrhoea. These methods are well reviewed and their value assessed.

Problems of typing and immunochemistry are reviewed next. The antigenic activity that can be ascribed to the major anatomical structures of the organism are defined and their complexity is discussed.

The interaction of gonococci with host cells and their resistance to phagocytosis are described and their significance revealed. *In vivo* and *in vitro* studies of gonococci are compared and contrasted. It is clear the gonococci grown *in vivo* are different morphologically, bacteriologically, and antigenically from gonococci grown *in vitro*. Efforts to bridge this gap are discussed.

The final chapters deal with treatment. One with the activity of trimethoprim and sulphamethoxazole against gonococci and the other with questions of resistance to antibiotics. The latter is of broad general interest.

This book is primarily of interest to the microbiologist but its wide coverage should make it worth reading for others interested in the developing aspects of sexually transmitted diseases.

H. S. K. Singha

Abstracts

These selected abstracts and titles from the world literature are arranged in the following sections:

Syphilis and other treponematoses

(Clinical and therapy; serology and biological false positive phenomenon; pathology and experimental)

Gonorrhoea

(Clinical; microbiology; therapy)

Non-specific genital infection

Reiter's disease

Trichomoniasis

Candidosis

Genital herpes

Other sexually transmitted diseases

Public health and social aspects

Miscellaneous

Syphilis and other treponematoses (Clinical and therapy)

Syphilitic meningitis with papilledema

G. M. TRENHOLME, A. A. HARRIS, P. P. MCKELLAR, J. E. JUPA, AND S. LEVIN (1977). *Southern Medical Journal*, 70, 1013-1014

Yaws in the Americas, 1950-1975

D. R. HOPKINS (1977). *Journal of Infectious Diseases*, 136, 548-554

Syphilis (Pathology and experimental)

Demonstration by labelled treponemal antigen of specific antibodies in the tissue infiltrates of secondary syphilis

K. SOLTANI, R. K. CHOY, AND A. L. LORINCZ (1977). *Journal of Investigative Dermatology*, 69, 439-441
A purified suspension of Nichols strain *Treponema pallidum* was disrupted ultrasonically and conjugated with fluorescein isothiocyanate. After absorption with normal skin tissue, dilutions were tested against cryostat sections of normal skin; a dilution of 1 in 10 was found to eliminate non-specific fluorescence.

Cryostat sections 6 µm in thickness from 12 biopsy specimens of skin lesions from 11 patients with proved secondary syphilis were stained with the conjugate. Sections of normal skin and various non-syphilitic lesions served as negative controls. Nine of the 12 syphilitic lesions showed granular patterns of fluorescence in the dermis in areas containing aggre-

gations of plasma cells; some fluorescence was directly located on these cells. No fluorescence was seen in the epidermis or dermal-epidermal junction, nor in the sections of non-syphilitic lesions. Prior treatment with unlabelled treponemal sonicate blocked fluorescence by the conjugated sonicate, thus demonstrating the specificity of the reaction. It is thought that the method may be of value in the tissue diagnosis of early syphilitic lesions which may mimic other skin disorders.

A. E. Wilkinson

In vitro cell response of *Treponema pallidum*-infected rabbits: I. Lymphocyte transformation

V. WICHER AND K. WICHER (1977). *Clinical and Experimental Immunology*, 24, 480-486

Transformation of peripheral lymphocytes from *Treponema pallidum*-infected rabbits was compared with the lymphocytes from appropriate controls. The lymphocyte response to phytohaemagglutinin was maximally suppressed in the infected rabbits approximately three weeks after development of a treponemal orchitis. In contrast there was a substantial spontaneous increase in the lymphocyte response after five days' incubation with lymphocytes taken from infected animals. A lesser degree of lymphocyte stimulation was detected using *Treponema pallidum*, Reiter strain, and cardiolipin antigens. It is suggested that the last response reflects stimulation of B lymphocytes, while the phytohaemagglutinin-induced depression of lymphocyte transformation represents either non-responsive T lymphocytes or

depletion of peripheral T lymphocytes resulting from the recruitment of the cells to the infected areas. Whether these changes denote immune paralysis or immune deviation remains speculative.

D. J. M. Wright

In vitro cell response of *Treponema pallidum*-infected rabbits. II. Inhibition of lymphocyte response to phytohaemagglutinin by serum of *T. pallidum*-infected rabbits

V. WICHER AND K. WICHER (1977). *Clinical and Experimental Immunology*, 29, 487-495

Serum inhibitors of lymphocyte response to phytohaemagglutinin and to the one-way allogenic mixed lymphocyte response were found in *Treponema pallidum*-infected rabbits. The inhibitors were non-dialysable and thermolabile (56°C for 30 minutes). No antibody-mediated lymphocytotoxicity could be demonstrated. Pooled sera taken from rabbits within the first six months of infection showed more inhibition of phytohaemagglutinin-induced lymphocyte transformation, the lymphocytes being normal rabbit lymphocytes, while with sera taken between one and two years after infection inhibition of phytohaemagglutinin lymphocyte transformation was less frequent and stimulation more common. In addition to inhibitors of lymphocyte transformation, lymphocytes from *Treponema pallidum*-infected rabbits seemed to possess an impaired response to phytohaemagglutinin lymphocyte stimulation. For problems relating to the detection of serum inhibitors, the original paper should be consulted.

D. J. M. Wright

In vitro cell response of *Treponema pallidum*-infected rabbits. III. Impairment in production of lymphocyte mitogenic factor

V. WICHER AND K. WICHER (1977).

Clinical and Experimental Immunology, 29, 496-500

The mitogenic activity on normal rabbit lymphocytes of supernatants from *Treponema pallidum*-infected rabbit inguinal and mesenteric lymph node cells and spleen cells were compared with supernatants from the appropriate controls. The antigen used was derived from Reiter strain spirochaetes. The active supernatants of lymph node cells of control animals showed a mitogenic index of between four and six and the infected animals of less than two. The active supernatant of spleen cells of infected and control rabbits showed a mitogenic index of less than two. It was suggested that the low mitogenic activity was due to the presence of inhibitors of DNA synthesis. This was reflected by the active supernatants depressing lymphocyte transformation to PPD when the lymphocytes were taken from BCG immunised animals and also by impairment of the allogenic mixed lymphocyte response.

D. J. M. Wright

Characterization of the attachment of *Treponema pallidum* (Nichols strain) to cultured mammalian cells and the potential relationship of attachment to pathogenicity

T. J. FITZGERALD, R. C. JOHNSON, J. N. MILLER, AND J. A. SYKES (1977). *Infection and Immunity*, 18, 467-486

Gonorrhoea (Clinical)

Gonorrhoea in preadolescent children: an enquiry into source of infection and mode of transmission

D. S. FOLLAND, R. E. BURKE, A. R. HINMAN, AND W. SCHAFFNER (1977). *Pediatrics*, 60, 153-156

From November 1974 until December 1975 a study was made of all reported cases of gonorrhoea in children under 10 years of age in Tennessee. Clinical manifestations of the 73 patients identified included vaginal infection (48), urethritis (11), conjunctivitis (8), and ophthalmia neonatorum (6). Specimens from a total of 203 relatives and associates of 54 patients were cultured. Fifty-four

(27%) had gonorrhoea; 43 of these were relatives. A history of sexual contact was found in 18 children, including seven for whom the contact had a positive culture for *Neisseria gonorrhoeae*. Sexual transmission was common in children with vaginitis or urethritis. In nine cases, sexual abuse or child neglect was suspected. The recognition of a child with gonococcal infection identifies a cluster of family members and associates who are at increased risk of having gonorrhoea.

Authors' summary

Gonococcal arthritis in pregnancy

A. MEHTA AND T. A. WRIGHT (1977).

Canadian Medical Association Journal, 117, 1190-1191

Gonorrhoea (Microbiology)

Transfer of plasmid-borne beta-lactamase in *Neisseria gonorrhoeae*

E. S. BARON, A. K. SAZ, D. J. KOPECKO, AND J. A. WOHLHIETER (1977).

Antimicrobial Agents and Chemotherapy, 12, 270-280

The recognition of penicillin resistance mediated by β -lactamase production in *Neisseria gonorrhoeae* has led to experiments to discover the nature and mode of transmission of the enzyme coding plasmid. This paper reports evidence for the transfer of β -lactamase mediated resistance by conjugation.

β -lactamase activity was detected by two methods, a quantitative microiodometric technique, and a qualitative dye technique. Transfer of resistance occurred with a frequency of 1.34×10^{-3} when a 100:1 ratio of donor to recipient organisms was used, but at only 1.04×10^{-4} with a ratio of 10:1. They were unable to effect resistance transfer at a ratio of 1:1. Loss of the resistance in recipients occurred at high frequency, consistent with plasmid-mediated resistance. The possibility that transfer could be by transformation was explored, and discounted by the inability of DNAase to reduce the frequency of transfer. Phage mediated transfer may also be a possibility, but there is no evidence as yet of a gonococcal phage. Evidence for conjugal transfer was seen by the inability of cell-free filtrates, or chloroform-treated donor cells to transfer the plasmid.

The plasmid complement of the strains was analysed by agarose gel

electrophoresis. Three plasmids were detected, 25 and 2.7 megadaltons (which are typical of previously described indigenous gonococcal plasmids), and a unique 4.8 megadalton plasmid found only in the β -lactamase producing strains. Consequently, it is surmised that 4.8 megadalton particle is the β -lactamase plasmid.

Transfer of the β -lactamase plasmid from one recipient to a second recipient could not be effected. This may have been due to the absence of the 25 megadalton plasmid from the donor recipient. It is therefore suggested that the 25 megadalton plasmid is responsible and necessary for the transfer of the 4.8 megadalton plasmid.

In conclusion, it is suggested that since the β -lactamase plasmid is readily lost, β -lactamase producing strains of *N. gonorrhoeae* are unlikely to become the predominant strains.

G. L. Ridgway

Gonorrhoea-diagnosis by Gram stain in female adolescent

E. R. WALD (1977). *American Journal of Diseases of Children*, 131, 1094-1096

Comparison of methods for rapid immunofluorescent staining of group-A streptococci and *Neisseria gonorrhoeae*

M. A. H. QURAISHI, W. WASHINGTON, AND S. L. ROSENTHAL (1977). *Health Laboratory Science*, 14, 279-281

Identification of *Neisseria* by electron capture gas-liquid chromatography of metabolites in a chemically defined growth medium

C. D. MORSE, J. B. BROOKS, AND D. S. KELLOGG, JR (1977). *Journal of Clinical Microbiology*, 6, 474-481

Place for gonorrhea serology

M. M. QUIGLEY (1977). *New York State Journal of Medicine*, 77, 2053

Gonorrhoea (Therapy)

The bactericidal action of spectinomycin on *Neisseria gonorrhoeae*

M. E. WARD (1977). *Journal of Antimicrobial Chemotherapy*, 3, 232-239

The *in vitro* bactericidal activity for gonococci of four different antibiotics—penicillin, tetracycline, kanamycin, and spectinomycin at their appropriate peak

serum concentrations was compared. Spectinomycin was much more rapidly bactericidal for gonococci than the other antibiotics tested; this contrasts sharply with the bacteriostatic action of spectinomycin for *Escherichia coli* at concentrations as high as 1 mg/ml. This rapid bactericidal action may be advantageous for any attempts to eradicate penicillinase producing gonococci. Electron microscope studies showed that spectinomycin produces alterations in gonococcal surface morphology leading to lysis. No significant differences were detected by sodium dodecyl sulphate polyacrylamide gel electrophoresis between the outer envelope proteins of gonococci grown in the presence or absence of spectinomycin, but there was evidence of ultrastructural damage to the cytoplasmic membrane. It is suggested that spectinomycin may inhibit the synthesis of a critical cytoplasmic membrane protein leading to ultimate impairment of the osmotic integrity of the cell.

Author's summary

The *in vitro* activity of 15 penicillins and mecillinam against *Neisseria gonorrhoeae*

B. A. WATTS AND I. PHILLIPS (1977).

Journal of Antimicrobial Chemotherapy, 3, 331-337

Minimum inhibitory concentrations (MICs) of 15 penicillins and mecillinam were determined on solid media for 92 strains of *Neisseria gonorrhoeae*. Regression lines for MICs of each antibiotic against those of benzylpenicillin were calculated. The order of activity against benzylpenicillin-insensitive strains was ampicillin, amoxycillin, azidocillin, ticarcillin, carbenicillin, sulfocillin, phenoxymethylpenicillin, methicillin, phenethicillin, mecillinam, propicillin, flucloxacillin, nafcillin, cloxacillin, and dicloxacillin. The regressions for flucloxacillin, cloxacillin, and dicloxacillin were indistinguishable as were those of azidocillin, ticarcillin, and carbenicillin. Only ampicillin and amoxycillin showed greater activity than benzylpenicillin against relatively resistant strains.

Authors' summary

Non-specific genital infection

An improved method for demonstrating the growth of chlamydiae in tissue culture

F. W. A. JOHNSON, L. Y. J. CHENCERELLE, AND D. HOBSON (1977).

Medical Laboratory Sciences, 35, 67-74

Coverslip cultures of McCoy cells were infected with the A22 strain of ewe abortion agent—a group B *Chlamydia*. Growth of the agent was enhanced by incorporating cycloheximide in the tissue culture medium throughout incubation of the infected monolayers. *Chlamydia* inclusions were better demonstrated by darkground microscopy of monolayers stained rapidly with Methylene Blue than by light microscopy of Giemsa-stained preparations. This simplified procedure has been applied to the diagnosis of human genital infections with *C. trachomatis*. An increased number of patients were found to have positive results, together with an increase in the number of chlamydial inclusion-forming units recovered from the swabs in cycloheximide-treated cultures.

Authors' summary

Cultivation of *Chlamydia trachomatis* in cycloheximide-treated McCoy cells

K. T. RIPA AND P.-A. MARDH (1977).

Journal of Clinical Microbiology, 6, 328-330

An isolation technique for *Chlamydia trachomatis* using McCoy cells is described. In contrast to earlier techniques employing such cells, no pretreatment of the cells was used. The glutarimide antibiotic cycloheximide was added to the culture medium used for incubating the cells after infection. Cycloheximide was used at concentrations that depressed, but did not completely inhibit, the metabolism of the eucaryotic host cells. In studies on different immunotypes of *C. trachomatis* cultured in the yolk sac of embryonated hen eggs, the cycloheximide technique was compared with a method using cells pretreated with 5-iodo-2-deoxyuridine. The cycloheximide method gave greater numbers of inclusion-forming units per coverslip for all the immunotypes of trachoma-inclusion conjunctivitis agents tested—that is A to I. In a study of 194 cervical and urethral specimens from women cycloheximide-treated McCoy cells were found to be more efficient than cells treated with 5-iodo-2-deoxyuridine for the isolation of *C. trachomatis*.

Authors' summary

Enzyme-linked immunosorbent assay for chlamydial antibodies

V. K. LEWIS, A. L. THACKER, AND

S. H. MITCHELL (1977). *Journal of Clinical Microbiology* 6, 507-510

An enzyme-linked immunosorbent assay (ELISA) detected chlamydial antibodies in human sera. The assay antigen produced in cell cultures infected with *Chlamydia psittaci* was formalin-fixed to microplates. Single convalescent-phase sera positive for chlamydial antibodies by a complement-fixation test were positive at even higher dilutions by ELISA. Paired sera with diagnostic rises in complement-fixing antibody showed seroconversion by ELISA also. Control sera from persons with no history of chlamydial infection were negative by both tests. Sera from patients with psittacosis or lymphogranuloma venereum were ELISA-positive, indicating that the assay with the antigen used in this study is genus specific rather than species specific.

Authors' summary

Bacteriology of the urethra in normal men and men with nongonococcal urethritis

W. R. BOWIE, H. M. POLLOCK,

P. S. FORSYTH, J. F. FLOYD,

E. R. ALEXANDER, S.-P. WANG, AND

K. K. HOLMES (1977). *Journal of Clinical Microbiology*, 6, 482-488

Sixty-nine Caucasian males without a previous history of urethritis and who developed non-gonococcal urethritis (NGU) and 39 similar men without urethritis (NU) were cultured from the urethra for *Chlamydia trachomatis*, *Mycoplasma hominis*, *Ureaplasma urealyticum*, aerobes, and anaerobes. *C. trachomatis* infection was proved by culture or serology in 26 (38%) of the NGU group and in one (3%) of the NU group; the *C. trachomatis*-negative NGU group had significantly more *U. urealyticum* (81%) than the *C. trachomatis*-positive NGU group (42%) or the NU group (59%). Aerobes were isolated from all but two men with *C. trachomatis*-negative NGU. Anaerobes were isolated from significantly more NU men (91%) than from men with NGU (66%). The aerobic and anaerobic flora of the two NGU groups were similar. The NU group had significantly more aerobic lactobacilli, *Haemophilus vaginalis*, alpha-haemolytic streptococci (not *Streptococcus faecalis*), and anaerobes, predominantly *Bacteroides* species. This study has provided information about the prevalence and the variety

of the aerobic and anaerobic microbiological flora of the anterior urethra of sexually active males. It does not implicate any bacteria other than *C. trachomatis* and *U. urealyticum* as potential causes of NGU.

Authors' summary

Interrelationship of *Chlamydia trachomatis* and other pathogens in the female genital tract

G. L. RIDGWAY AND J. D. ORIEL (1977). *Journal of Clinical Pathology*, 30, 933-936

The isolation of *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Trichomonas vaginalis*, and *Candida albicans* in the female genital tract was studied in 1323 patients attending a venereal disease clinic. Disruption of the cell monolayers used for the isolation of *C. trachomatis* was significantly associated with the presence of *T. vaginalis*; this effect was markedly reduced by the addition of vancomycin to gentamicin and amphotericin B in the transport and growth media. The only significant positive association was the more frequent isolation of *C. trachomatis* in the presence of *N. gonorrhoeae*. There was a negative association between *N. gonorrhoeae* and *C. albicans* and between *T. vaginalis* and *C. albicans*, the fungus being isolated significantly less often when these microorganisms were present.

Authors' summary

***Chlamydia* and non-specific urethritis**

J. W. SEGURA, T. F. SMITH, L. A. WEED, AND G. R. PETTERSEN (1977). *Journal of Urology*, 117, 720

Reiter's disease

The sacroiliitis of acute Reiter's syndrome
A. S. RUSSELL, P. DAVIS, J. S. PERCY, AND B. C. LENTLE (1977). *Journal of Rheumatology*, 4, 293-296

Thirty-three patients with the classical triad of Reiter's syndrome were examined for sacroiliitis by bone scan. HLA typing was carried out and found to be positive for HLA-27 in 29 patients. Sacroiliitis was definitely present in nine and probably present in 15 patients. Asymmetry was prominent as noted by earlier investigators in contrast with the symmetrical picture associated with ankylosing spondylitis. As the abstractor

observed some years ago, the sacroiliitis settled usually when the disease remitted and persistence of sacroiliitis was rare.

G. W. Csonka

Acute venereal arthritis—comparative study of acute Reiter syndrome and acute gonococcal arthritis

W. C. MCCORD AND K. M. NIES (1977). *Archives of Internal Medicine*, 137, 858-862

Trichomoniasis

The effect of trichomonal vaginitis on vaginal pH

C. L. PARSONS, S. LOFLAND, AND S. G. MULHOLLAND (1977).

Journal of Urology, 118, 621-622

Assuming that the vaginal pH is the primary defence in premenopausal women against colonisation of the vaginal introitus with Gram-negative organisms, the authors studied normal women and those with trichomonal vaginitis to determine whether inflammation of the vaginal tissues had a significant effect on the pH.

Midstream specimens of urine and cultures from the periurethral area and vaginal introitus were taken from 16 premenopausal women with no history of urinary tract infection and from six premenopausal women with trichomonal vaginitis. Fourteen of the 16 normal women had a vaginal pH of 4.0 or less and a predominantly Gram-positive introital flora. In contrast, five of the six women with vaginitis had a vaginal pH of 5.0 or more. The introital flora in these patients were similar to those of the control group, but unfortunately five of them had completed courses of ampicillin for cystitis about one week before entering this study. The sixth patient also had cystitis with a heavy growth of *Escherichia coli* in the vagina, urethra, and midstream urine. Her vaginal pH was 5.1. This dropped to 4.0 after a week's course of metronidazole and nitrofurantoin. The perineal flora was Gram-positive by then.

From these findings the authors suggest that inflammation of vaginal tissues results in an increase in the pH of the vaginal secretions. This leads to colonisation of the vaginal introitus favouring the onset of cystitis.

C. S. Ratnatunga

An evaluation of thidazole as single-dose therapy for the treatment of *Trichomonas vaginalis*

R. JONES AND P. ENDERS (1977). *Medical Journal of Australia*, 2, 679-681

Candidosis

The susceptibility of *Candida albicans* to amphotericin B, nystatin, and 5-fluorocytosine

L. K. HAMRA AND I. J. PANKIEWICZ (1977). *Medical Journal of Australia*, 2, 749-752

Genital herpes

Correspondence: The prevalence of *Herpesvirus hominis* in genital lesions with suggestive chancre morphology

N. SHARON (1977). *American Journal of Clinical Pathology*, 68, 628-629

For a period of two years 73 university students were referred for darkground examination of genital lesions for the presence of *Treponema pallidum*. Specimens of exudate were also cultured for *Herpesvirus hominis* in HEP-2 cell cultures and on the chorioallantoic membranes of 10-day-old fertile eggs.

T. pallidum was found in three of 59 lesions in males and in two of 14 in females. *Herpesvirus hominis* was isolated from 22 darkground negative lesions in 51 males and from four of 11 lesions in females. It is recommended that virus isolation techniques should form part of the routine investigation of genital lesions.

A. E. Wilkinson

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Incidence and distribution of herpes simplex virus Types 1 and 2 from genital lesions in college women

J. E. KALINYAK, G. FLEAGLE, AND J. J. DOCHERTY (1977). *Journal of Medical Virology*, 1, 175-181

During a nine-month period, 9772 women were treated at the student health centre's gynaecology clinic. Herpes simplex virus was isolated from 30 of 57 patients clinically diagnosed as suffer-

ing from a herpetic or herpetic-like genital infection, giving a virological incidence rate of 0.31%. Using virus plaque diameter in chick embryo cells and heat stability of viral thymidine kinase, 37% of the isolates were classified as herpes simplex virus Type 1 and 63% were classified as herpes virus Type 2.

Authors' summary

Antibody activity to Type 1 and Type 2 herpes simplex virus in human cervical mucus

B. M. COUGHLAN AND G. R. B. SKINNER (1977). *British Journal of Obstetrics and Gynaecology*, **84**, 622-629

Cervical mucus collected from 29 patients randomly selected from a routine gynaecological clinic was examined for neutralising antibody activity to herpes simplex virus Types 1 and 2. Findings were correlated with the neutralising activity against these viruses found in sera from the same patients. The cervixes of the patients were all macroscopically normal, and no patient had clinical or laboratory evidence of genital tract infection.

Neutralising antibody to Type 1 herpes simplex virus was detected in 24 of 28 patients and to Type 2 herpes simplex virus in 18 of 24 patients. This neutralising antibody was present in both IgA and IgG fractions of cervical mucus, the IgA fraction containing about three times more neutralising antibody per unit of immunoglobulin concentration than the IgG fraction. Studies on concentrated pooled mucus samples identified a type-common immunoprecipitin, in addition to type-specific neutralising antibodies against both virus types. There was on average a higher Type 2 neutralising antibody activity in mucus than in serum, and there was no correlation between serum and mucus neutralising antibody levels for either virus type.

The authors feel their results support the concept of an independent local antibody system in the cervix. They suggest local antibodies may provide a more sensitive index of previous exposure to herpes simplex Type 2 than circulating antibodies. Studies on neutralising antibody activity to herpes simplex virus may thus strengthen the existing seroepidemiological evidence of an association between herpes simplex virus Type 2 and carcinoma of the cervix.

Shirley Richmond

Latent herpes simplex virus infections in sensory ganglia of mice after topical treatment with adenine arabinoside and adenine arabinoside monophosphate

R. J. KLEIN AND A. E. FRIEDMAN-KIEN (1977). *Antimicrobial Agents and Chemotherapy*, **12**, 577-581

Herpes simplex virus vaccines

T. G. WISE, P. R. PAVAN, AND F. A. ENNIS (1977). *Journal of Infectious Diseases*, **136**, 706-711

Other sexually transmitted diseases

Chancroid: a study of 500 cases

T. TAN, V. S. RAJAN, S. L. KOE, N. J. TAN, B. H. TAN, AND A. J. GOH (1977). *Asian Journal of Infectious Diseases*, **1**, 27-28

Chancroid has become rare in Western countries but it is still prevalent in tropical and subtropical areas. Five hundred cases seen during a period of 12 months at the Middle Road Hospital, Singapore, are reviewed. Exudate from the lesions was examined by Gram-stained smears for the presence of organisms resembling *Haemophilus ducreyi* and cultured for 48 hours in the patient's own serum after this had been inactivated at 56°C for 30 minutes; 278 specimens gave positive results by both methods. Sixty-two per cent of the patients had single and 38% multiple lesions; pain and adenitis were present in 19%. Sixty-four (13%) patients also had other venereal diseases including 21 with syphilis and 13 with gonorrhoea. Treatment with tetracycline (2 g daily for two weeks) was effective in 189 patients. Many infections were resistant to tetracycline but responded well to a course of 7-14 g streptomycin. *A. E. Wilkinson*

[Reprinted from *Abstracts on Hygiene*, by permission of the Editor.]

Correspondence: spread of condylomata acuminata to infants and children

F. J. STORRS (letter) and L. GOLDMAN (reply) (1977). *Archives of Dermatology*, **113**, 1294

Condyloma acuminatum of scrotum

B. RAY (1977). *Journal of Urology*, **117**, 739

Thio-tepa in the management of anorectal condylomata acuminata: report of two cases

M. D. KERSTEIN (1977). *Diseases of the Colon and Rectum*, **20**, 625-626

Miscellaneous

Pathogenesis of acute pelvic inflammatory disease: role of contraception and other risk factors

D. A. ESCHENBACH, J. P. HARNISCH, AND K. K. HOLMES (1977). *American Journal of Obstetrics and Gynecology*, **128**, 838-850

In a case-control of matched pairs, the risk of acute pelvic inflammatory disease (PID) was 4.4 times higher in users of intrauterine devices (IUDs) than in non-users ($P < 0.001$). Of approximately 500 000 cases of acute PID occurring annually in the United States, an estimated 110 000 are attributable to IUDs, costing over \$44 million a year. PID was attributable to the IUD in 77% of IUD users. No particular type of IUD was implicated. The relative risk of acute PID in IUD users compared with non-users was higher in nulligravid women than in previously pregnant women and was directly related to socioeconomic state (SES), but the total annual risk of PID in IUD users appears inversely related to SES. IUD use significantly increased the risk of non-gonococcal PID. Fever occurred in 13 (21%) of 61 IUD users and in 59 (41%) of 143 non-users ($P < 0.025$). Among women with non-gonococcal PID, an adnexal mass was noted in 14 (40%) of 35 IUD users and in only 12 (15%) of 78 non-users ($P < 0.01$). An increased risk of gonococcal PID was found in non-Caucasians and in women not using contraception, while the risk of non-gonococcal PID was increased in women with a history of gonorrhoea. Oral contraceptives may protect women with gonorrhoea from developing PID. Menstruation precipitates the onset of symptoms of gonococcal PID.

Authors' summary

Amoebiasis: an increasing problem among homosexuals in New York City

M. J. SCHMERIN, A. GELSTON, AND T. C. JONES (1977). *Journal of the American Medical Association*, **238**, 1386-1387

The authors report the case histories of two male homosexual patients attending

the New York Hospital in whose stools *Entamoeba histolytica* was found during the investigation of attacks of lower abdominal pain and watery stools. Both patients also had positive stool cultures for *Shigella flexneri*. As neither patient had travelled from New York City the authors postulate oroanal transmission of the pathogens. One patient's sexual consort was also found to have *E. histolytica* and *Sh. flexneri*. The amoebiasis responded to treatment with diiodohydroxyquin and metronidazole, and the shigellosis to ampicillin.

The authors then reviewed the records of the 98 patients in whose stools *E. histolytica* had been isolated in the previous five years. Fifty-six were males over the age of 15 but only 50 could be studied. Thirty of these males had travelled in areas where *E. histolytica* is endemic, most of them during the previous year. Two of these travellers and all 20 of the remainder were found to be homosexual. Other venereal diseases had occurred in these 20 males; syphilis in five, gonorrhoea in six, hepatitis in nine, shigellosis in six, and giardiasis in five. The authors also note that the incidence of amoebiasis in homosexuals is increasing.

G. D. Morrison

Venereal transmission of enteric pathogens in male homosexuals

D. MILDREAN, A. M. GELB, AND D. WILLIAM (1977). *Journal of the American Medical Association*, **238**,

1387-1389

The authors report the case histories of two male homosexual patients seen at the Beth Israel Medical Centre New York.

The first patient had a rapid onset of watery diarrhoea, chills, and fever, although his stools had been looser and more frequent than usual during the previous two months. Stool examination showed *Giardia lamblia* and culture showed *Shigella sonnei*. There had been recent sexual contact with a male having a history of amoebiasis. He was treated with quinacrine for his giardiasis and rapidly recovered. He remained well for six months, and then developed abdominal pain and watery diarrhoea. Stool examination showed *E. histolytica*, but no other pathogen. He was treated with metronidazole and rapidly recovered. During the six-month period he had frequent sexual intercourse with other men.

The second patient gave a history of successful treatment for amoebiasis three years earlier. Eight months before he was seen, he developed fever, nausea, and bloody diarrhoea. Stool examination revealed *Giardia lamblia* and *E. histolytica*, but the stool was not cultured for bacteria. Despite antiprotozoal treatment the bloody diarrhoea continued and *Shigella sonnei* was cultured from his stools. He was treated with ampicillin but his symptoms continued. Three stool examinations and cultures were free of pathogens but the diarrhoea continued until he was treated with metronidazole.

The authors discuss the incidence of amoebiasis and giardiasis in homosexual males in the USA. They conclude that, analingus with possible coprophagy is the likely means of transmission of all three pathogens.

G. D. Morrison

Examining the homosexual male for sexually transmitted diseases

Y. M. FELMAN AND J. M. MORRISON (1977). *Journal of the American Medical Association*, **238**, 2046-2047

Bacteriological findings in cultures of clinical material from Bartholin's abscess

M. W. D. WREN (1977). *Journal of Clinical Pathology*, **30**, 1025-1027

Side effects of minocycline: different dosage regimes

D. W. GUMP, T. ASHIKAGA, T. J. FINK, AND A. M. RADIN (1977). *Antimicrobial Agents and Chemotherapy*, **12**, 642-646

Rheumatoid factor in a patient with Reiter's disease and aortic incompetence

R. M. DU BOIS AND S. FREEDMAN (1977). *British Medical Journal*, **2**, 1260-1261

Treatment of Behçet's syndrome with transfer factor

R. E. WOLF, H. H. FUNDENBERG, T. M. WELCH, L. E. SPITLER, AND M. ZIFF (1977). *Journal of the American Medical Association*, **238**, 869-871



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27th October	Short papers.
24th November	Herpes Genitalis.
26th January 7.00 p.m.	} Etiology and Prognosis of Reiter's Disease. Inherited Susceptibility to Reiter's Disease.
30th March	
27th April	Early Syphilis—the pros and cons of epidemiological treatment.
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A journal of the sexually transmitted diseases and allied disorders edited for the Medical Society for the Study of Venereal Diseases

Contents

Sexually transmitted diseases on a tropical island

V. S. RAJAN (Singapore) *page 141*

Cell-mediated immunity during syphilis. A review

C. S. PAVIA, J. D. FOLDS, AND J. B. BASEMAN (USA) *page 144*

TPHA test. Experience at the Clinic of Dermatology, University of Milan

E. ALESSI AND L. SCIOCCATI (Italy) *page 151*

Improved management in the diagnosis of gonorrhoea in women

A. E. JEPHCOTT AND S. RASHID (Gt Britain) *page 155*

Studies on *Neisseria gonorrhoeae* cultured in liquid medium

J. A. EAVES (Gt Britain) *page 160*

The efficacy of cefuroxime for the treatment of acute gonorrhoea in men

J. D. PRICE AND J. L. FLUKER (Gt Britain) *page 165*

Absence of detectable local antibody in genitourinary tract secretions of male contacts of women infected with *Trichomonas vaginalis*

J. P. ACKERS, R. D. CATTERALL, W. H. R. LUMSDEN, AND A. MCMILLAN (Gt Britain) *page 168*

Specific immunity to human papilloma virus (HPV) in patients with genital warts

J. VIAC, M. J. STAQUET, M. MIGUET, M. CHABANON, AND J. THIVOLET (France) *page 172*

Recurrent genital candidosis in women and the effect of intermittent prophylactic treatment

F. DAVIDSON AND R. F. MOULD (Gt Britain) *page 176*

Clotrimazole (Canesten) in the treatment of candidal balanitis in men

M. A. WAUGH, E. G. V. EVANS, K. C. NÄYYAR, AND R. FONG (Gt Britain) *page 184*

Measuring the outcome of contact tracing. 1: A description of the patient and contact populations studied

A. SATIN AND A. MILLS (Gt Britain) *page 187*

Measuring the outcome of contact tracing. 2: The responsibilities of the health worker and the outcome of contact investigations

A. MILLS AND A. SATIN (Gt Britain) *page 192*

A survey of attitudes of patients in a department of genitourinary medicine

M. J. BALSDON, B. CHARLTON, M. CURRAN, AND H. S. K. SINGHA (Gt Britain) *page 199*

Which patients with sexually transmitted diseases default?

Report of a survey in one clinic

S. PERFREMENT AND K. OVERFIELD (Gt Britain) *page 201*

Correspondence *page 205*

Notice *page 206*

Book reviews *page 207*

Abstracts *page 209*

August 1978 Vol. 54 No. 4

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British Journal of Venereal Diseases

BRITISH MEDICAL ASSOCIATION TAVISTOCK SQUARE LONDON WC1

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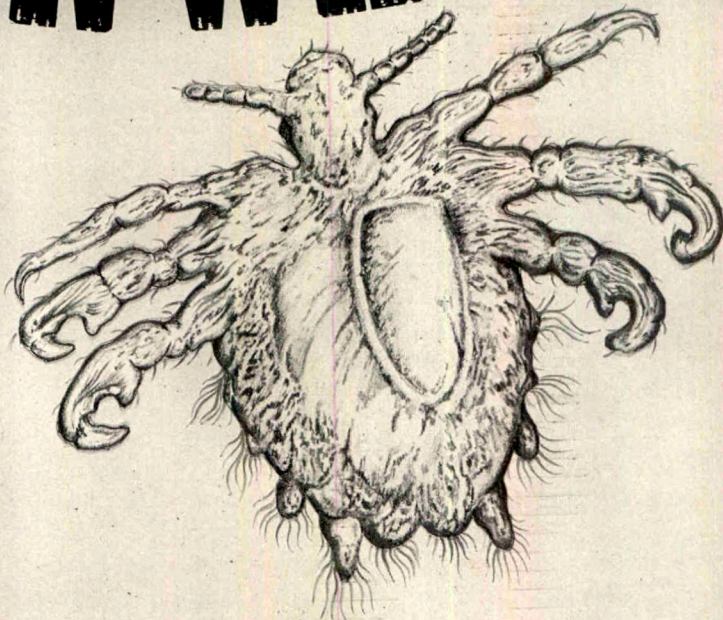
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Sexually transmitted diseases in Surinam

Observations and thoughts

H. E. MENKE*

From the Dermatological Service, Surinam

Introduction

Surinam, a tropical country, is geographically located in the north-eastern part of South America and bordered by Guiana, French Guiana, Brasil, and the Atlantic Ocean (see Figure). The country became a republic on 25 November 1975 after 300 years of colonisation by the Netherlands. Surinam is sparsely populated with 400 000 inhabitants, approximately 60% of whom live in the capital Paramaribo and its surrounding areas. The remainder of the population is concentrated in the coastal plain and fewer than 10% live in the interior. About 130 000 people of Surinamese origin live in Holland. Most of them moved there shortly before Surinam gained independence and there is close contact between them and their home country.

Surinam, apart from the Amerindians, are descendants of voluntary and involuntary immigrants. However, Surinam is distinct in having a more heterogeneous population, originating from Africa, Asia (Indonesia, India, and China), and Europe. These groups practise several religions including Hinduism, Islam, various Christian denominations, and Animism. Although there is substantial cultural intermingling and assimilation, most ethnic groups have retained features of their original culture. In relation to this, one can find differences in the distribution of diseases in the various ethnic groups.

The economy of Surinam is based primarily on the production and export of bauxite, alumina hydroxide, alumina, and timber (plywood) together with agricultural products (rice and bananas).



Figure Geographical position of Surinam.

Historically and sociologically, Surinam has a closer relationship with countries of the Caribbean area than with Latin American countries. As in other countries in the Americas the inhabitants of

For almost a century the country has been self-sufficient in general medical practitioners trained at the local medical school founded in 1882. Recently, this medical school was incorporated as a faculty of the University of Surinam, which was founded in 1968. Medical specialists are educated abroad, mainly in the Netherlands. Unlike many developing nations, the physician to population ratio in Surinam

*Present address and address for reprints: H. E. Menke, National Institute of Public Health, POB 1, Bilthoven, The Netherlands

Received for publication 8 January 1978

is good, with one doctor for every 2000 inhabitants. Medical research, which is partially done in conjunction with foreign universities and international institutions, is developing. Most research is focused on tropical infectious and parasitic diseases of high morbidity—such as schistosomiasis, filariasis, leprosy, and treponemal diseases.

Control of STD

Sexually transmitted disease (STD) comes under the jurisdiction of the Ministry of Health, and its control is implemented by the dermatological service. This service was founded in 1971 by Dr Paul Niemel, a dermatovenereologist, who is currently the director. There is an outpatient department in Paramaribo, and rural areas have consultation services and special campaigns. Before 1971 any patient suffering from STD, leprosy, or a dermatological disorder had to visit one of three different medical departments. These departments are now integrated into one service. It is therefore more difficult for patients to be identified by laymen as suffering from specific diseases and there is less stigma.

Control of STD and leprosy has been helped by the fact that patients are treated free of charge in the outpatient department. However, many patients with STD prefer to consult physicians in private practice. Although in theory reporting is compulsory, doctors are reluctant to report cases; therefore, the statistics are unreliable. Self medication is often practised by the patients.

Incidence of STD

Clinical observations suggest there has been a gradual increase in the number of patients with STD in Surinam during the last 15 years although the actual number visiting the dermatological service outpatient department with STD has remained stable during the last four years that records have been kept. Data on the five classical venereal diseases indicate that gonorrhoea, early infectious syphilis, and donovanosis are much more common than lymphogranuloma venereum and chancroid. Donovanosis is diagnosed more often than it was a decade ago. This is attributed to cases in people who moved from the interior to Paramaribo. Contrary to western European countries and North America, non-specific urethritis is diagnosed less frequently than gonorrhoea.

Positive results to serological tests for treponemal diseases are common. Among 2395 adult outpatients seen between 1 January and 1 September 1977, a total number of 685 (30%) had a reactive VDRL (Venereal Disease Research Laboratory)

test. These patients were from urban and rural areas. Further analysis with treponemal tests indicates that biological false positive VDRL reactors are rare. The blood transfusion service shows that many people in Surinam have been exposed to treponemal infection as about 5% of the aspirant donors (mostly urban dwellers) have a reactive VDRL test.

A recent study in the central northern part of the country revealed that out of 2971 people 13.3% reacted positively to the VDRL test (treponemal tests were also positive). For clinical and epidemiological reasons, the reactive VDRL tests were attributed to yaws. It is interesting to note that classical symptoms of yaws were not seen; the cases were either mild or entirely asymptomatic (attenuated treponematoses). In the interior we detected a much higher seropositive ratio. For instance of 408 people examined in one village half showed a reactive VDRL test. Moreover cases of yaws in the interior are generally of the classical type, in contrast to the attenuated cases in the central-northern region.

The data indicate that a substantial part of the Surinamese population has been exposed to treponemal infections. Clinical syphilis is regularly diagnosed in the urban area; cases of yaws from the rural areas are less common. Pinta has not been identified.

Prostitution

The influx of relatively large amounts of money, including revenues from the bauxite industry and development aid, probably contributes to the recent increase in prostitution. Because of economic progress, more sailors, technicians, and businessmen, mostly unattached, visit Surinam for short periods and form a flourishing clientele for the prostitutes. These women can be categorised as indigenous or migratory; the latter constitutes a fast growing group and their number is now estimated to be 300. They originate from the Caribbean islands and South America, and travel from country to country within this part of the world, which indicates a pattern of regionalisation of their profession (regional prostitution). Moreover, there is indication of a transatlantic movement of these prostitutes to European countries and back to the Americas (transatlantic prostitution).

The government has no clear policy on prostitution. By establishing reasonable rapport with the prostitutes and hotel managers and others concerned, some control has been instituted and prostitutes are examined voluntarily every two weeks and a presumptive treatment is administered.

In the interest of public health it is important to know to what extent the migratory prostitutes import and export STD and help in the spread of disease. However, since they are examined neither when entering the country nor when leaving, no quantitative assessment can be made on the extent of disease transmission across borders. We interviewed 273 men with gonorrhoea between January and September 1977 to obtain information on the source of the disease. Migratory and indigenous prostitutes were the sources in 12% and 17% of cases respectively; the remaining 71% indicated other types of contacts. The figures suggest that prostitutes as well as the general population may be a significant reservoir of disease. However, insufficient data limit the conclusions that can be drawn.

A high percentage of positive results to serological tests for syphilis was found in the migratory prostitutes. Out of 135 prostitutes examined between January and September 1977, 41% of them showed a reactive VDRL and/or rapid plasma reagin (RPR) card test. Treponemal tests gave positive results in most VDRL and RPR reactive cases. VDRL titres were generally low and clinical treponemal disease was not identified. The positive serological results can be related to venereal syphilis as well as to non-venereal treponematoses.

Comment

In spite of the small amount of detailed data presented on sexually transmitted diseases in Surinam, a general impression can be ascertained.

The similarity in social structure and culture makes it probable that there is a close similarity between the STD pattern in Surinam and that in other Caribbean countries.

European doctors may encounter medical problems in people originating from Surinam but living in Holland that are alien. For example, positive results to serological tests for syphilis in Surinamese expatriates may be caused by previous yaws infection. It is obvious that the movement of people between Europe and the Caribbean area can introduce unexpected pathogens and diseases in either region.

International prostitution, a phenomenon which has so far received insufficient attention, transmits diseases across borders. The extent of the problem, however, is largely unknown. If a booklet, such as the one used by sailors, were introduced which gives coded information on diagnosis, laboratory results, and treatment, a contribution might be made to our knowledge and control of STD internationally.

I thank Dr Paul Niemel, dermatovenereologist and Dr Ernest Guile, MPH for their advice.

Attachment of virulent *Treponema pallidum* to human mononuclear phagocytes

BARRY D. BRAUSE AND RICHARD B. ROBERTS

From the Division of Infectious Diseases, Department of Medicine,
Cornell University Medical College, New York, USA

SUMMARY The predominant phagocyte in established human syphilitic lesions is the mononuclear phagocyte. As these leucocytes may be important in immunity to *Treponema pallidum*, the initial interaction between human phagocytes and pathogenic *T. pallidum* (Nichols strain) was studied *in vitro*. Motile, virulent *T. pallidum* attach to the surface membrane of phagocytes but are not ingested by these cells. Heated, non-motile treponemes are not cell-associated but are observed free in the extracellular medium. Attachment is polar, at one or both terminal portions of the treponeme, and is neither serum- nor complement-dependent. Ingestion of virulent treponemes was not observed by phase-contrast or by electron microscopy in the presence of normal human or rabbit serum and complement. Techniques were chosen to preserve both the fragile surface constituents of the treponeme and the phagocytic function of mononuclear cells. Unfixed preparations were observed by phase-contrast microscopical examination during incubation to differentiate motile from non-motile organisms and fixed preparations were used for quantitation of attachment. This model should be useful for studying humoral and cell-mediated immunity in syphilis.

Introduction

The inflammatory response in human syphilis is characterised by plasma cells, macrophages, and lymphocytes (Robbins, 1967). Furthermore, the principal phagocytic cell in histological sections of syphilitic chancres, condylomata lata, the maculopapular rash of secondary syphilis and in gummas is the mononuclear phagocyte. Previous studies of non-pathogenic *Treponema refringens*-rabbit peritoneal macrophage interactions revealed intracellular treponemes within vacuoles (Musher *et al.*, 1972). However, the initial interaction between virulent viable *Treponema pallidum* and animal or human monocytes has not been previously reported.

The complex process of phagocytosis may be divided into three sequential steps: attachment of the microbe to the surface membrane of the phagocytic cells, ingestion of the attached microbe, and digestion. Knowledge of these biological processes in *T. pallidum*-phagocyte interactions would greatly help our understanding of the pathophysiology of syphilis in humans. This report describes the initial

interaction or attachment *in vitro* of *T. pallidum* to human mononuclear leucocytes.

Materials and methods

PREPARATION OF *T. PALLIDUM*

Virulent *T. pallidum* (Nichols strain) obtained from the laboratory of Dr Paul H. Hardy, Johns Hopkins Medical Institutions, was used. The organism was cultivated in the testes of Venereal Disease Research Laboratory-negative (VDRL-negative), New Zealand white rabbits. An inoculum of 10^6 treponemes in 10% heat-inactivated rabbit serum (Microbiological Associates, Inc., Bethesda, Maryland) produced a palpable orchitis in 9-11 days when rabbits were housed in air-conditioned quarters (15-17°C) and were given intramuscular hydrocortisone sodium phosphate (Merck and Co., West Point, Pennsylvania), 6 mg/kg daily starting 48 hours after inoculation. Within two days of the development of fulminant orchitis, animals were killed with pentobarbitone sodium (50 mg/kg, i.v.) and the testes removed aseptically. Each testis was sliced with sterile scissors, and tissue sections were vortexed intermittently for 20 minutes in 1.5 ml of RPMI-1640 tissue culture medium without antibiotics (Grand Island Biological Co., Grand Island,

Address for reprints: Dr B. D. Brause, Division of Infectious Diseases, Cornell University Medical College, New York, NY 10021, USA

Received for publication 21 December 1977

NY). The extraction mixture was then centrifuged at 1200 g for 10 minutes to sediment gross tissue debris, testicular cells, and spermatozoa, and the concentration of treponemes in the supernate adjusted to 5×10^7 – 2×10^8 treponemes per millilitre (Magnuson *et al.*, 1948). Treponemes obtained by this aerobic extraction method were 99–100% actively motile and retained their motility and infectivity for at least three hours in an aerobic atmosphere. Studies were also performed using non-motile treponemes which had been heated at 56°C for five minutes.

Treponemes were also harvested by an anaerobic technique incorporating the motility-sustaining medium used in the *Treponema pallidum* immobilisation (TPI) test described by Nelson and Diesendruck (1951) and modified by Metzger *et al.* (1961). This medium was further modified by the use of RPMI-1640 tissue culture medium instead of 0.85% NaCl as the gelatin solvent. Whereas the aerobic extraction method took only 20 minutes, this technique took two hours.

PREPARATION OF HUMAN MONONUCLEAR CELLS

Heparinised peripheral blood was pipetted on to Ficoll (Pharmacia Chemicals, Uppsala, Sweden)—Hypaque (Winthrop Laboratories, New York, NY) gradients (24 parts of 9% Ficoll to 10 parts of 50% Hypaque), using a modification of the method of Gordon *et al.* (1974). Sterile plastic materials were used throughout the procedure. The gradient was centrifuged for 40 minutes at 220 g. A central zone containing phagocytes, lymphocytes, and platelets was removed, and the cells were washed three times with 10 ml phosphate-buffered saline (calcium- and magnesium-free) containing 1 ml EDTA (0.15 mol/l) per 500 ml. After centrifugation for 10 minutes at 340 g, the pellet was resuspended in the desired volume of RPMI-1640 tissue culture medium (without antibiotics) containing 5% heat-inactivated human serum to obtain a final concentration of 5×10^6 cells (phagocytes and lymphocytes) per millilitre. Aliquots containing 0.2 ml of the cell suspension were then placed on sterile 15 mm round glass coverslips in 10 × 35 mm Petri dishes and incubated in 5% CO₂/95% air for 90 minutes at 37°C. Phagocytes adhered to the glass surface, while lymphocytes and platelets were washed off with additional tissue culture medium. The monolayers were then stored in serum-free RPMI-1640 tissue culture medium at 37°C in 5% CO₂ for varying times ranging from 15 minutes to 14 days before use. Culture medium was changed every 48 hours.

SERUM

Normal rabbit serum was collected from VDRL-negative animals. Human serum was obtained from VDRL-negative volunteers who had no history of syphilis. Human serum and human phagocytes were autologous. All sera were used immediately or stored in small aliquots at –65°C. Serum was frozen once and thawed immediately before each experiment. In certain experiments, serum was heat-inactivated at 56°C for 30 minutes.

T. PALLIDUM-HUMAN MONOCYTE INTERACTIONS

Glass coverslips with adherent human phagocytes were placed in cylindrical vials. Suspensions containing 2.5 – 5×10^7 treponemes in tissue culture medium were added in 0.5 ml amounts to mononuclear cell monolayers with or without 20% fresh or heat-inactivated normal human or rabbit serum. Certain mixtures also contained 0.025 ml guinea-pig complement (Grand Island Biological Co., Grand Island, NY) which represented approximately 700 log units/ml (Hederstedt, 1961).

To ensure that testicular preparations did not interfere with phagocytosis, control incubations with inert, ingestible polystyrene latex particles (Dow Chemicals, Indianapolis, Indiana) with a 1.099 µm diameter were used in all experiments (Griffin and Silverstein, 1974). Control mixtures contained 0.5 ml of the treponeme suspension with or without serum and 10⁸ latex particles. Serum was added in 0.1 ml volumes to comprise 20% of the incubating mixtures, and latex particles represented 0.05 ml of the total volume.

MICROSCOPY

Initially, unfixed, wet preparations of *T. pallidum* phagocyte mixtures were examined at a magnification of ×756 in a phase-contrast oil immersion system for periods of two to three hours at 37°C in air. Preparations were made by inversion of coverslips with adherent cells on to approximately 3 µl of treponeme suspension. Coverslip edges were sealed with paraffin.

Treponeme-phagocyte mixtures were also incubated at 37°C in 5% CO₂/95% air. After 60 minutes the coverslips with adherent phagocytes and treponemes were washed with RPMI-1640 tissue culture medium and fixed with 2.5% glutaraldehyde in 0.1 mol/l sodium cacodylate buffer (pH 7.40). Coverslips were mounted and sealed on glass slides for phase-contrast microscopical examination. Quantitative evaluations were made a few hours after fixation by determining the numbers of cell-attached and unattached organisms per 50 treponemes. Calculated percentages of attached treponemes repre-

sented the proportion of the total number of treponemes remaining on the glass coverslip after incubation, washing, and fixation, which were adherent to leucocyte cell surfaces (Table 1). The density of phagocytes and treponemes per coverslip was determined by multiplying the mean number of phagocytes or treponemes per $\times 756$ microscopic field by 4.4×10^3 . The microscopic field area was 0.04 mm^2 , the coverslip area 177 mm^2 . Generally there were 3.12×10^4 cells per coverslip, and the average density of treponemes per coverslip was 2.82×10^4 for virulent motile treponemes and 1.52×10^4 for heated, non-motile treponemes.

Results

MORPHOLOGICAL OBSERVATIONS DURING INCUBATION

The interaction between virulent *T. pallidum* (Nichols strain) and human phagocytes was first

studied using unfixed, wet preparations. Examination by phase-contrast at 37°C showed that treponemes attached themselves to the cell surface. Attachment was observed within five minutes of incubation and was polar with one or both terminal portions of the treponeme in contact with the cell surface (Fig. 1). Although firmly attached at one end to the phagocyte, treponemes retained their characteristic motility with both spiral and flexion movements. Motility was maintained for at least $2\frac{1}{2}$ hours in an aerobic atmosphere. Heat-killed, non-motile treponemes did not attach to the cell surface but remained free in the surrounding medium.

The morphology of human monocytes derived from peripheral blood changed during *in vitro* cultivation on a glass surface. At first adherent cells were rounded with a relatively small surface area. After 24 hours the cells resembled tissue macro-

Table 1 Attachment of virulent, motile *T. pallidum* (Tp) to human mononuclear leucocytes with or without normal serum

	Aerobic extraction of Tp		TPI test extraction of Tp	
	% attached Tp*	% cells with attached Tp	% attached Tp*	% cells with attached Tp
No serum	89 (75-98)	48 (38-63)	93 (92-94)	39 (35-42)
20% heat-inactivated normal serum				
Human	85 (75-92)	46 (38-72)	91 (89-94)	35 (32-39)
Rabbit	91 (73-97)	48 (32-69)	87 (83-90)	39 (38-40)

Values represent the mean and range of three to five experiments

*Percentage of the total number of treponemes remaining on the glass coverslip after incubation, washing, and fixation adhering to leucocyte cell surfaces

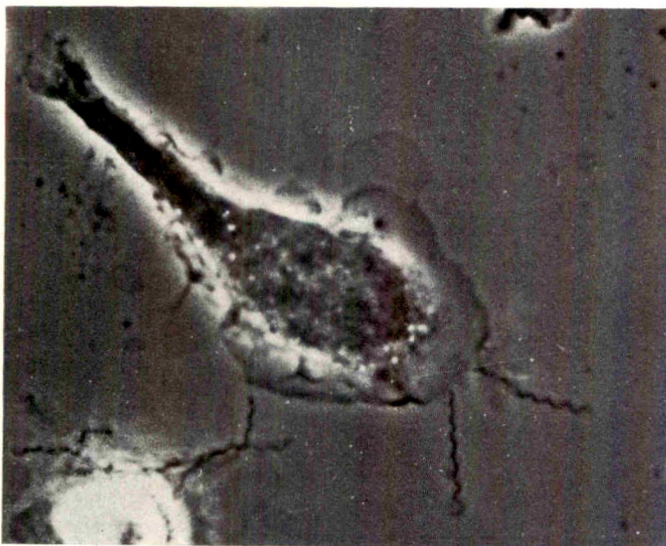


Fig. 1 Polar attachment of *T. pallidum* to surface membrane of a human mononuclear phagocyte under phase-contrast microscopy ($\times 1260$).

phages with spreading of the cytoplasm and the presence of a ruffled membrane on the cell surface. The cytoplasm continued to spread with longer durations of cultivation. The nucleus and prominent nucleoli remained unchanged. During the 2–3 hour incubation of phagocytes with treponeme suspensions no morphological changes were seen—that is, vacuolisation did not occur and changes did not appear in the ruffled membrane. Treponemes attached themselves in a similar manner to those rounded mononuclear cells which were maintained on glass coverslips for 15 minutes as well as to those with large cytoplasmic surfaces which had been incubated for 14 days. Phagocytes were routinely cultivated *in vitro* for 2–3 days before incubation with suspensions of treponemes (Fig. 1). Attachment of *T. pallidum* was also observed at the surfaces of rabbit peripheral mononuclear leucocytes and unstimulated mouse peritoneal macrophages.

Ingestion of *T. pallidum* was not seen even when individual attached treponemes were observed for as long as 40 minutes with or without 20% fresh or heat-inactivated normal human or rabbit serum. In addition, heat-killed, non-motile organisms were not ingested under similar experimental conditions. Ingestion of polystyrene latex particles was observed under all of these conditions.

MORPHOLOGICAL OBSERVATIONS AFTER FIXATION

The frequency of attachment was quantitated using glutaraldehyde-fixed preparations after 60 minutes of aerobic incubation. As shown in Table 1, 79–98% (mean of 89%) of treponemes extracted by either

the aerobic or the TPI test technique were attached to phagocytes with or without 20% heat-inactivated normal human or rabbit serum ($P > 0.05$).^{*} As previously stated the percentages of attached treponemes represent that proportion of the total number of treponemes remaining on the glass coverslip which were adherent to cell surfaces after incubation, washing, and fixation. Almost half the phagocytes had one treponeme or more attached to the cell surface. The treponeme density approximated that of the macrophages in these fixed preparations. No intracellular organism was detected by phase-contrast microscopy.

A control incubation mixture containing leucocytes, aerobically-prepared treponemes, and latex particles was included in all experiments to show that the treponeme suspension did not inhibit phagocytosis. Numerous intracellular latex particles were observed in the presence of attached treponemes (Fig. 2). Latex particles were added to preparations at the beginning or 15 minutes before the end of incubation. Over 90% of phagocytes ingested latex particles under either of these conditions indicating that the phagocytic function of the cells was intact. Treponemes harvested by the standard anaerobic method of the TPI test were also incubated with macrophages and latex particles. The results of these experiments performed in triplicate showed that whereas the technique of extraction had no effect on the frequency of attachment of treponemes, the ingestion of latex particles was inhibited suggesting that the TPI test medium

^{*}Statistical analysis was by a *t* test with 25 degrees of freedom where $t = 0.3686$

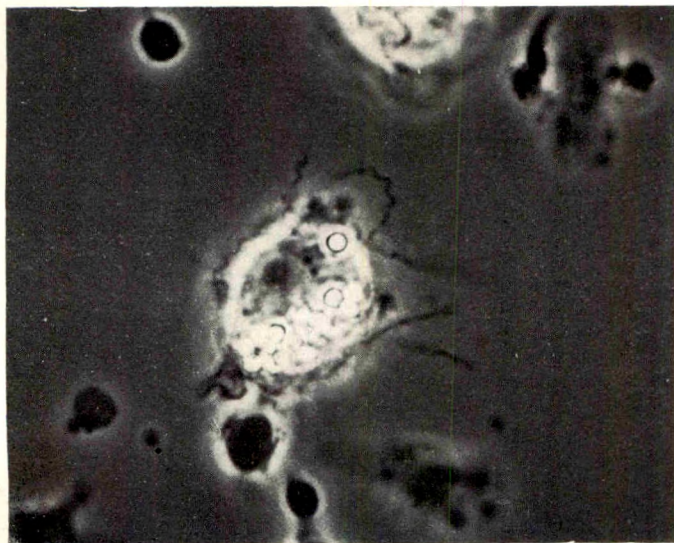


Fig. 2 Intracellular latex particles in a human mononuclear phagocyte with attached *T. pallidum* by phase-contrast microscopy. Note horse-shoe configuration of one treponeme attached at both ends to the cell surface ($\times 1008$).

may interfere with phagocytosis. Furthermore, phagocytes incubated with latex particles suspended in TPI test medium (without treponemes) failed to ingest the latex particles. TPI test medium was therefore not used in subsequent studies.

As *T. pallidum* in the absence of serum and complement attached to the cell surface regardless of the extraction procedure (Table 1), attachment was not due to complement-dependent immune adherence (Nelson, 1953). However, complement in association with antibody present in the testicular preparation or in normal serum may be required for the ingestion of attached organisms. The presence of fresh sera or complement had no effect on attachment ($P > 0.05$)† (Table 2). In addition, intracellular treponemes were not observed in preparations containing fresh serum or heated-inactivated serum supplemented with guinea-pig complement.

Table 2 *Effect of complement on the attachment of virulent, motile T. pallidum (Tp) to human mononuclear leucocytes*

	% attached Tp*	% cells with attached Tp
Complement only	98 (96-100)	42 (37-47)
20% heat-inactivated normal serum		
Human with complement	87 (72-98)	38 (26-46)
Rabbit with complement	94 (79-100)	38 (25-56)
20% fresh normal serum		
Human	97 (96-100)	42 (36-52)
Rabbit	93 (88-96)	33 (24-39)

Values represent the mean and range of five experiments

*Percentage of the total number of treponemes remaining on the glass coverslip after incubation, washing and fixation adhering to leucocyte cell surfaces

When heated, non-motile treponemes were incubated with phagocytes only 14% of treponemes appeared to be attached to cells (Table 3). Normal human or rabbit serum and complement did not increase the frequency of attachment. The frequency of attachment was less than 6% when incubation was performed on a mechanical shaking device to compensate for absence of motility in the heated treponeme. The small percentage of heated, non-motile treponemes which appeared to be attached to cells in fixed preparations may not actually be adherent to the cell surface as no attachment was observed during incubations with unfixed preparations. In wet, unfixed preparations ingestion of heated, non-motile treponemes was not observed.

†*t* test with 24 degrees of freedom where $t = 1.3620$

Table 3 *Attachment of heated, non-motile T. pallidum (Tp) to human mononuclear leucocytes with or without normal serum and complement*

	% attached Tp*	% cells with attached Tp
No serum or complement	14 (12-16)	7 (4-9)
20% heat-inactivated normal serum		
Human with complement	8 (6-11)	4 (3-5)
Rabbit with complement	5 (4-7)	2 (2-3)

Values represent the mean and range of three experiments

*Percentage of the total number of treponemes remaining on the glass coverslip after incubation, washing and fixation adhering to leucocyte cell surfaces

To show that treponeme suspensions from rabbit testes did not inhibit phagocytosis suspensions other than polystyrene latex were added to the incubation mixtures. Thus, heat-killed staphylococci, viable *Toxoplasma gondii*, and zymosan were all ingested by phagocytes whereas *T. pallidum* remained attached to the cell surface.

Ingested *T. pallidum* were not seen in any of the preparations. However, since intracellular treponemes may not be readily identifiable by phase-contrast microscopy, electron microscopy was performed on preparations in which 100% of the phagocytes showed attached treponemes in the presence of 20% heat-inactivated normal human serum and guinea-pig complement. For this it was necessary to use treponeme concentrations of 2×10^8 per ml. No intracellular treponemes were observed in such preparations.

Discussion

Studies of the immunobiology of *T. pallidum* have been hindered by the inability to cultivate the virulent treponeme *in vitro*, by damage to the organism by extraction procedures, and by the effect of substrates such as extraction medium ingredients and rabbit testicular debris on *in-vitro* preparations. Techniques established in this laboratory have been used in an effort to preserve the fragile surface constituents of the treponeme and the *in-vitro* function of mononuclear phagocytes. The organism was extracted rapidly from rabbit testicular slices and subjected to a short centrifugation only to avoid trauma before use in phagocyte preparations. Thus treponemes were incubated with phagocytes only 30 minutes after slicing the rabbit syphilitic testes in contrast* to the more lengthy anaerobic TPI test extraction technique. The extraction medium, RPMI-1640, which is a tissue culture medium without antibiotics, or

reducing agents, sustained aerobic treponemal motility for at least three hours and was employed routinely for *in-vitro* macrophage cultivation in preference to the TPI test medium. The system is simple, reproducible, and convenient. Only a few treponemes in the suspension remained on the monolayer after they had been incubated, washed, and fixed, probably because of their motility and small mass. Recently techniques have been developed in this laboratory in which *T. pallidum* is centrifuged directly on to cell monolayers (unpublished data).

Previous investigators have shown that virulent *T. pallidum* may adhere to a variety of surfaces including normal human erythrocytes and eukaryotic cells in tissue culture (Nelson, 1953; Fitzgerald *et al.*, 1975). By contrast, heated, non-motile treponemes and avirulent, cultivatable treponemes (*Treponema denticola* and *Treponema phagedenis* biotype Reiter) do not adhere to cultured rabbit testicular cells in tissue culture (Fitzgerald *et al.*, 1976).

We have demonstrated that *T. pallidum* also adheres to the surface of human mononuclear phagocytes as well as those of the rabbit and mouse. Attachment is not complement-mediated as described for other particles requiring specific receptor sites (Mantovani *et al.*, 1972). Attachment is also not due to 'immune adherence', as described by Nelson, since the treponeme extracted by TPI test methods does not require complement or syphilitic serum for adherence to cell surfaces (Nelson, 1953). Heated, non-motile treponemes do not attach to human mononuclear phagocytes. Preliminary studies in our laboratory indicate that non-pathogenic treponemes (*T. phagedenis* biotype Reiter and *T. refringens*) also do not attach to mononuclear phagocytes *in vitro*. These findings suggest that specific surface constituents on virulent, motile treponemes may be important in the attachment process but this adherence is non-specific since these organisms may also attach to a variety of substrates, including normal human erythrocytes and eukaryotic cells in tissue culture (Nelson, 1953; Fitzgerald *et al.*, 1975).

As shown in these studies, attachment is polar with the terminal portion only of the treponeme adhering to the cell surface. Morphological studies have shown that the terminal portions of treponemes differ from the rest of the body (Hovind-Hougen, 1976). Electron microscopy with negative staining has shown that the terminal portions of treponemes have three zones of varying density, whereas the remainder of the organism is relatively homogeneous. Their shape may be species-specific. Thus avirulent treponemes cultivated in broth have tapered or blunt ends whereas pathogenic treponemes have

pointed ends. The relationship between these morphological differences and attachment awaits further study.

Attachment of virulent, motile treponemes to phagocytic cells without subsequent ingestion suggests that virulent *T. pallidum* may have anti-phagocytic surface factors. These observations are consistent with previous electron microscopical examinations of human syphilitic chancres in which treponemes were mainly extracellular, although a few intracellular treponemes were seen mainly in non-phagocytic cells (Azar *et al.*, 1970; Sykes *et al.*, 1974). These studies, however, described fixed specimens in which the motility and viability of intracellular or extracellular organisms could not be assessed. Thus, the few intracellular treponemes identified in these reports may not have been fully intact at the time of their ingestion. Since treponeme viability cannot be quantitated by *in-vitro* cultivation at the present time, it is important that studies of treponeme-phagocyte interaction include unfixed preparations to differentiate motile from non-motile organisms.

The initial interaction of *T. pallidum* with human mononuclear phagocytes *in vitro* demonstrates that virulent, motile treponemes attach to the surface membrane but are not ingested by these cells. Studies are now in progress to examine subsequent interactions of these organisms with phagocytic leucocytes.

This investigation was supported by Training Grant No. AI 00465 and Research Grant No. AI 12932 from the National Institutes of Health.

We thank Ms Patricia DeNunzio for her technical assistance and Dr Thomas C. Jones, for his advice and assistance in electron microscopy.

The authors are indebted to Dr Paul H. Hardy, Jr. of the Johns Hopkins Medical Institutions for his advice and co-operation in the cultivation and study of virulent *Treponema pallidum*.

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Lack of proliferation of circulating white blood cells in patients with syphilis before and after a Jarisch-Herxheimer reaction

MAIJA HORSMANHEIMO

From the Department of Dermatology, University Central Hospital, Helsinki, Finland

SUMMARY The occurrence of lymphoblasts in peripheral blood, measured by incorporating tritiated thymidine ([³H]dThd), was studied before, during, and after a Jarisch-Herxheimer reaction in patients with early syphilis. No difference in [³H]dThd incorporation into peripheral white blood cells was found between five patients with syphilis and 19 healthy controls, whereas in 20 cord blood samples [³H]dThd incorporation was significantly increased.

Introduction

The pathogenesis of the Jarisch-Herxheimer reaction (JHR) remains obscure. It has been suggested that the JHR is caused by the reaction of treponemal breakdown substances with sensitised syphilitic tissue (Sheldon and Heyman, 1949) or by the release of leucocyte endogenous pyrogen or other plasma kinins by treponemal substances (Schofield *et al.*, 1968). Although the release of histamine is not an important factor in the symptoms of JHR (Heyman *et al.*, 1952; Zachariae and Nielsen, 1974), it has been suggested that cell-mediated immune mechanisms play a role, and many reports have documented the importance of cell-mediated immune reactions in syphilis (Festenstien, *et al.*, 1967; Bădănoiu *et al.*, 1969; Levene *et al.*, 1969, 1971; Fulford and Brostoff, 1972; Wright and Grimble, 1974; Musher *et al.*, 1974, 1975; From *et al.*, 1976).

Small numbers of immature, proliferating white blood cells can be found in the peripheral blood of healthy subjects. Increased numbers of proliferating mononuclear leucocytes are seen in the blood in a wide variety of infections (Lalla, 1974). In addition, a proliferative response of circulating blood cells has been reported after immunisation, after virus vaccination, and after clinically positive provocation tests to the drugs which previously had caused rashes in the same patients (Lalla, 1974). The size of this response has been found to be related to the strength of the antigenic stimulus.

The present study was undertaken to investigate whether proliferating white blood cells could be found in the peripheral blood of patients with syphilis.

Materials and methods

Three men (aged 18, 23, and 47 years) and two women (aged 21 and 22 years) from the Department of Dermatology, University Central Hospital, Helsinki, were studied. They were all in hospital and suffering from early syphilis, two in the primary stage and three in the secondary stage. All gave positive results by standard serological tests for syphilis. In addition, treponemes (*Treponema pallidum*) were found in the primary chancres of the two patients with primary stage and in one of the patients with secondary stage syphilis.

All patients received 600 000 units of sodium penicillin in the morning, and features of the JHR were observed each hour until the fever had subsided. The patients did not receive any other drug. The highest temperatures during the JHR in these patients were 39.5, 38.6, 38, 37.8, and 37.3°C. A macular rash appeared in one of the patients during the JHR, two patients claimed headache, and one had pain in his enlarged inguinal lymph nodes. After the JHR, all patients continued to be treated with 600 000 units a day of procaine penicillin.

The control series comprised 19 age-matched healthy volunteers (employees of the University Central Hospital). As a positive control, 20 samples of cord blood were also studied. Cord blood was obtained from the Department of Obstetrics and Gynaecology after normal deliveries.

Address for reprints: Maija Horsmanheimo, MD, Department of Dermatology, University Central Hospital, Snellmaninkatu 14, SF-00170 Helsinki 17, Finland

Received for publication 3 January 1978

Two millilitres of peripheral venous blood or cord blood was withdrawn into a prewarmed (37°C) syringe containing 0.5 ml of 6% dextran, 50 iu heparin, and 2.5 μ Ci tritiated thymidine ([3H]dThd, specific activity 6.7 Ci/mmol) (New England Nuclear Corporation). After incubation for an hour at 37°C the buffy coats were collected. The cells were counted in a haemocytometer. Cell preparations were made with a cytocentrifuge (Shandon Scientific Co. Ltd, London) and were stained with May-Grunwald-Giemsa (MGG) stain. Two aliquots of 0.2 ml were immediately chilled and used for scintillation counting (Packard, Model No. 3375). The results for tritiated thymidine incorporation into DNA are given as counts per minute per 10⁶ white blood cells.

Results

Before the study began, the optimal incubation time of the cells with [3H]dThd was studied. Incubation for one hour at 37°C was found to be a reliable labelling time in controls and cord blood. Longer incubation did not significantly increase the incorporation of isotope into the cells. As in previous studies (Lalla, 1974), blast responses were studied on the fifth day. [3H]dThd incorporation before the JHR was 49 ± 33 (mean $\pm$ SD) in patients with syphilis, 31 ± 16 in 19 controls, and 323 ± 208 in 20 cord blood samples (Figure). After the JHR, [3H]dThd incorporation was 59 ± 26 in the patients with syphilis, which did not differ significantly from the values before JHR or from those of the controls.

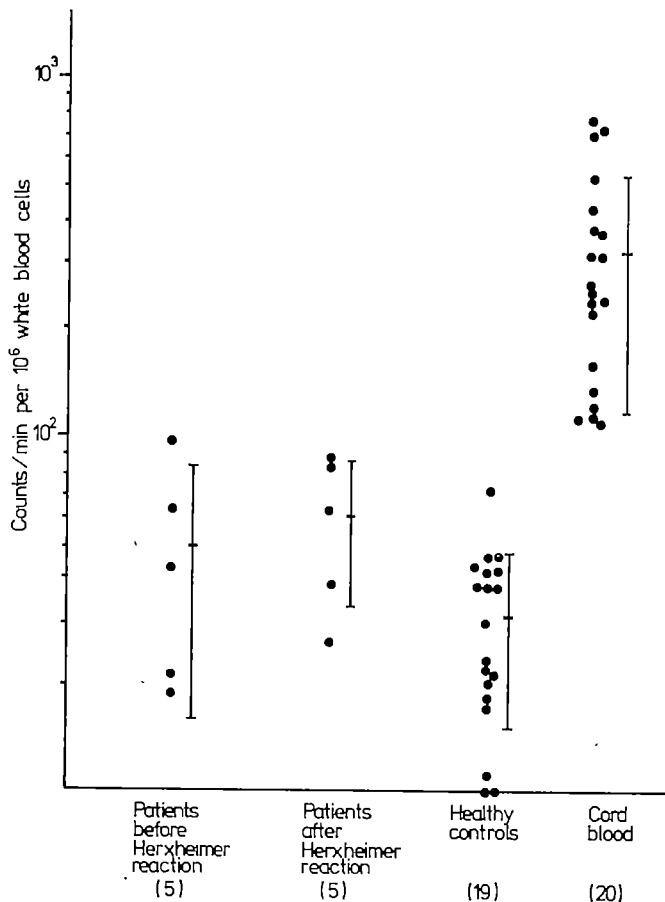


Figure [3H]dThd incorporation into white blood cells in patients with early syphilis before and after Jarisch-Herxheimer reaction, in healthy controls, and in cord blood samples.

[3H]dThd incorporation in cord blood was significantly higher than in adults ($p < 0.001$ by Student's *t* test). In addition, in one of the patients, incorporation was measured after eight hours, when the fever had reached its maximum, and in two patients after one day of the penicillin dose. In one patient incorporation was measured after two days, in two patients after three days, and in one after four days; the values did not differ significantly from those on the fifth day.

Discussion

The number of proliferating cells in the peripheral blood increases in a variety of infections, after immunisation, and during the course of some other diseases in which immunological mechanisms play a central role (Lalla, 1974). Parallel *in vivo* and *in vitro* studies with bone marrow indicate that the *in vitro* results within the first hour of incubation with [3H]dThd are identical with those obtained *in vivo*, when bone marrow cells are removed one hour after injection of the label (Bond *et al.*, 1959; Rubini *et al.*, 1960). Therefore, incorporation within the first hour *in vitro* seems to reflect the *in vivo* capacity of the cells for DNA synthesis. The advantage of the present method is that the separation of buffy coat cells and incorporation of [3H]dThd takes place simultaneously and immediately after the withdrawal of the peripheral blood cells.

The timing of the lymphoblast response to antigenic stimulation has already been studied in healthy volunteers after immunisation with bacterial vaccines and after stimulation with four antigens combined (Gump and Fekety, 1967; Crowther *et al.*, 1969). The peak proliferative response was between five and seven days later. In cutaneous hypersensitivity reactions after clinically positive provocation test with sulphonamides and some other drugs, the maximum blood lymphoblast proliferation *in vivo* was observed five days after the test (Lalla and Virolainen, 1974). Five days were also required for the optimal lymphocyte transformation response to antigens *in vitro* (Horsmanheimo and Virolainen, 1974). Thus, this *in vivo* lymphocyte transformation is probably analogous to the antigen-induced transformation of specific lymphocytes *in vitro* (Crowther *et al.*, 1969; Virolainen *et al.*, 1974).

In healthy adults, a very low level of [3H]dThd incorporation was found in the present study, in accord with previous studies (Lalla, 1974). In addition, large numbers of DNA-synthesising cells were found in cord blood samples, as has also been reported previously (Lalla, 1974). DNA-synthesising cells in cord blood have been shown to be pre-

dominantly labelled myeloid or erythroid cells, and only small numbers of lymphoblasts have been found (Lalla, 1975). The increase in the number of lymphoblasts takes place between four and seven days after birth (Lalla, 1975) and probably represents a response of the lymphoid system to the enormous number of new antigens met by the organism.

The lack of [3H]dThd incorporation by peripheral blood cells of patients with early syphilis indicates that stimulation of peripheral blood lymphocytes by *T. pallidum* antigen occurs neither in early syphilis nor after JHR, in which large amounts of treponemal breakdown substances are released. The results with this new method support the findings of some previous studies dealing with cell-mediated immunity in the early stage of syphilis. Musher *et al.* (1974) found that cell-mediated hypersensitivity skin reaction to treponemal antigens was negative in the primary stage of syphilis, in contrast to the latent stage in which it became positive. Furthermore, inhibition of leucocyte migration in the presence of proteins from Reiter treponemes has been demonstrated in the late latent stage of the disease, whereas leucocyte migration is normal or even enhanced in patients with primary syphilis (Fulford and Brostoff, 1972). In the lymphocyte transformation test, the treponemal antigen and Reiter protein-induced blast response have been found to be lowest in early primary syphilis (Bădănoiu *et al.*, 1969; Fulford and Brostoff, 1972). In addition, Levene *et al.* (1969) found a significant reduction in lymphocyte transformation in the presence of phytohaemagglutinin (PHA) in patients with primary and secondary syphilis, and plasma from patients with secondary syphilis suppressed transformation of normal lymphocytes. Since the *in vivo* blast response in peripheral blood may be related to the *in vitro* transformation of sensitised lymphocytes in response to the specific antigen, it is suggested that the same mechanisms resulting in the inhibition of lymphocyte transformation *in vitro* in early syphilis may be responsible for the lack of [3H]dThd incorporation by peripheral blood cells from patients with syphilis demonstrated in this study. This reduction of lymphocyte reactivity could be caused by an excess of treponemal antigen, antigen-antibody immune complexes, a circulating antilymphocyte antibody, or by some other inhibitory factor(s).

The lack of [3H]dThd incorporation by peripheral blood cells during and after the Jarisch-Herxheimer reaction does not support the possibility that this reaction is the result of an immunisation state induced by treponemal antigen. However, the existence of inhibitory mechanisms responsible for the lack of blast responses cannot be ruled out.

Assistance by Charles L. Smith in preparing the manuscript is gratefully acknowledged.

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Lymphocyte reactivity in patients with gonococcal urethritis

LILI ROSENTHAL AND ERIC SANDSTRÖM

From the Transplantation Immunology Laboratory, Huddinge Hospital, Huddinge, the Department of Rheumatology, Karolinska sjukhuset, and the Department of Dermatovenereology, Karolinska Institutet at Södersjukhuset, Stockholm, Sweden

SUMMARY Lymphocyte reactivity to virulent gonococcal antigen T₂ and the non-pathogenic *Neisseria pharyngis* (NPN) has been studied by using the ¹⁴C-thymidine uptake in cell cultures from 42 patients with gonococcal urethritis and from 18 controls. The DNA synthesis in cell cultures with T₂ antigen was higher in 21 female patients than in the 18 controls. No differences in DNA synthesis were observed in antigen-stimulated cell cultures from patients with single or multiple infections, from patients with urogenital complications, or from controls. Gonococcal antibodies in the serum were detected by the gonococcal complement-fixation test (GCFT). A study of the possible correlation between the outcome of the serological test and the cellular response to gonococcal antigen showed that ¹⁴C-thymidine uptake in lymphocyte cultures from male patients with negative GCFT, stimulated with T₂ antigen, was much lower than the thymidine uptake in stimulated cell cultures from all the other male and female patients ($P < 0.001$). The DNA synthesis was higher in cell cultures from seronegative women than from seronegative men ($P < 0.01$). A significant difference ($P < 0.01$) was also noted in the lymphocyte reactivity to gonococcal antigen between controls and all patients, except in those men who gave negative results to the serological tests. There were no differences between these two groups with respect to the thymidine uptake in NPN-stimulated cell cultures.

Introduction

Various immunological techniques to detect serum antibodies in gonococcal infection have been described. The gonococcal complement-fixation test (GCFT) is the most widely used test in the serodiagnosis of gonorrhoea (Sandström and Danielsson, 1975), and the only method used routinely. With this test sera from about 30% of patients with uncomplicated genital gonorrhoea and 50% of patients with salpingitis and genital gonococcal infections give positive results in a single serum specimen (Danielsson *et al.*, 1972). Non-specific reactions are found in about 5% of uninfected patients but are of little importance in patients with venereal diseases (Magnusson and Kjellander, 1965).

The cellular response to gonococcal antigen in patients with gonococcal urethritis has been studied *in vitro* using the lymphocyte transformation test

(Kraus *et al.*, 1970; Esquenazi and Streitfeld, 1973; Grimble and McIlmurray, 1973; Kearns *et al.*, 1973). This cellular response was not demonstrable in cells from controls but it was present in varying degrees in patients suffering from gonococcal urethritis. Antigen-induced lymphocyte transformation was only slightly greater in patients with a first infection than in controls, while the difference was often significant between patients with a history of at least two gonococcal infections and controls (Kearns *et al.*, 1973). In 1972 Rosenthal found no differences in lymphocyte reactivity to gonococcal antigen between patients with newly contracted gonorrhoea and controls. In 1975 it was demonstrated (Rosenthal, 1975) that lymphocyte stimulation to gonococcal antigen occurred in patients with joint complications after gonococcal infection, while no lymphocyte stimulation could be demonstrated in patients with gonococcal urethritis (either single or multiple infections) or in the controls.

The present investigation was undertaken to find out whether the discrepancy between our results

Address for reprints: L. Rosenthal, Department of Rheumatology, Karolinska Sjukhuset, 10401 Stockholm, Sweden

Received for publication 17 October 1977

and those of previously published studies may be due to the composition of the material or to the involvement of the urogenital tract in the form of prostatitis or salpingitis. The object was also to study the correlation between both the cellular and the humoral immune response.

Materials and methods

PATIENTS AND CONTROLS

Forty-two Caucasian patients were investigated; of these 27 were considered to have uncomplicated gonococcal urethritis and 15 had gonococcal urethritis with urogenital complications. Seventeen of the 27 patients (11 women and six men) had an infection for the first time which will here be referred to as single infection, and 10 had had multiple infections. Of the latter 10 patients, eight (two women and six men) had had only one previous episode of uncomplicated gonococcal infection. This episode had occurred between four months and seven years before examination. One man had had two previous gonococcal infections, and another had had several infections during a period of three and five years, respectively. The mean age of these 27 patients was 22.9 years; for the women it was 20.5 and for the men 25.3 years. The duration of symptoms was short (one and three days) in two of 13 female patients. Three women visited the hospital as contacts of patients with gonorrhoea. The other eight women had had a discharge for several months before the diagnosis was established.

Of 14 men, six with single gonococcal infection had had symptoms lasting a few days; one of these was symptom-free at the time of investigation. In the other eight men, who had had multiple gonococcal infections, the history of the current infection was also short, varying between one day and three weeks. Blood specimens from the patients with uncomplicated gonorrhoea were obtained at the time of diagnosis.

Of the above-mentioned 42 patients, 15 had local complications. Of these, eight were women with salpingitis (mean age 21.4), and seven were men with prostatitis (mean age 24.9). Seven of the women with salpingitis, who had cervical cultures positive for gonococci, had been referred from the gynaecological department with this diagnosis. Five of the eight women had salpingitis for the first time, and three had had previous episodes thought to be caused by gonococci. The interval between the diagnosis of infection and investigation was 33.9 ± 21.9 days. The duration of symptoms was short (less than one month) in six patients, whereas it was two months in the remaining patient.

Five of the seven male patients were diagnosed as having acute prostatitis because of localised swelling and tenderness of the prostate gland on rectal palpation. These changes disappeared after treatment. The duration of symptoms in all these cases was less than one month. Two men had had chronic prostatitis for the last two and three years.

The mean interval between the diagnosis of current infection and investigation was 26 days with a range of 0-90 days. Blood specimens from the patients with urogenital complications were obtained in the convalescent period of the disease (after antibiotic treatment) in 12 out of 15 patients. In all cases but one the diagnosis of gonococcal infection rested on the culture of oxidase-positive, Gram-negative diplococci fermenting glucose but not maltose or laevulose. The remaining patient, a man, was diagnosed by the finding of intracellular diplococci in a smear of urethral pus, stained with methylene blue.

Eighteen blood donors, 13 men and five women, served as controls. Data on age and race were available for 13 men and two women, with a mean age of 38.5 and 25.0 years, respectively. The controls were all Caucasian.

GONOCOCCAL COMPLEMENT-FIXATION TEST

Sera were collected at the same time as blood sampling for the lymphocyte transformation test and were stored frozen at -20°C until tested. Sera were thawed, inactivated at 56°C for 30 minutes, and analysed by the micromodification of the laboratory branch complement fixation test (US Public Health Service, 1965) as modified by Danielsson *et al.* (1972). Antigen was produced from T₂ colonies of five strains collected in 1973 from patients with uncomplicated gonococcal infection and grown on gonococcal special agar for 22 hours at 36°C in 5% CO_2 atmosphere (Sandström and Danielsson, 1977). Each of the strains was collected in saline, adjusted to a concentration of 25 mg/ml wet weight of bacterial cells in veronal buffer, heated to 60°C for 30 minutes, and tested in checkerboard titrations for anticomplementary activity and optimal dilution against a human serum with a known titre. The strains could be mixed in equal proportions to a final concentration of 1.0 mg/ml. The dilution of a serum specimen that gave an inhibition of haemolysis of 70% or more was regarded as the final titre. Such an inhibition at a serum dilution of 1:5 was regarded as a positive reaction.

LYMPHOCYTE TRANSFORMATION TEST

Lymphocyte separation

Lymphocytes were isolated from heparinised venous blood by flotation of blood on Ficoll-Isopaque (Lymphoprep, Nyco, Oslo, Norway), as described by Bøyum (1968).

The interface cell layer was removed and washed twice in phosphate-buffered saline (PBS). The cells were resuspended in Eagle's medium, in Earle's solution (Flow Laboratories, Irvine, Scotland) containing glutamine (2 mmol/l), 100 iu/ml penicillin, and 100 µg/ml streptomycin. To this medium was added 10 mmol/l N-2-hydroxy-ethylpiperazine-N'-2-ethane sulphonic acid (HEPES) buffer (Flow Laboratories). For induction of DNA synthesis the medium was supplemented with nonessential amino-acids and pyruvate as described by Mishell and Dutton (1967). The cells were cultured in this medium, with 10% heat-inactivated human AB serum added. Triplicate cell cultures were set up in plastic tissue culture tubes (no. 2058 Falcon Plastics) containing 0.5 ml lymphocyte suspension (3×10^6 cells/tube). To the cell cultures 0.05 ml of the following bacterial antigens and mitogen were admixed: *Neisseria gonorrhoeae* (strain 819) of colony morphology type T₂ and *Neisseria pharyngis* (strain 6131) at a concentration of approximately 2×10^8 organisms/tube, 0.05 ml of PPD tuberculin, 0.01 mg/ml and 0.05 ml of phytohaemagglutinin (PHA) 1:100. A control culture without antigen was made in each case. The culture tubes were incubated at 37°C in humidified 5% CO₂ in air atmosphere.

Measurements of DNA synthesis

A concentration of 0.1 µCi ¹⁴C-thymidine (specific radioactivity 25 mCi/mmol per litre, the Radiochemical Centre, Amersham) in 0.05 ml of PBS was added to the cultures with antigens and unstimulated cultures on day 5 and to cell cultures with PHA on day 2. The cultures were harvested 24 hours later using distilled water, and the cells were collected on Sartorius membrane filters. The filters were placed in scintillation plastic vials and left to dry at room temperature overnight. Five millilitres of toluene-based scintillation fluid were added to each vial, and the radioactivity was measured in a Intertechnique (Nanoteknik, Sweden) scintillation counter. The results were expressed as arithmetic means of counts/min of triplicate tubes $\pm$ SE.

BACTERIAL ANTIGENS

The following bacterial strains were used: one strain of *N. gonorrhoeae* (laboratory designation 819) whole cells of colony morphology type T₂ (virulent) (Kellogg *et al.*, 1963) and one strain of *N. pharyngis* (designation 6131) whole cells. The

bacterial antigens were prepared by Dan Danielsson, Örebro, Sweden. The procedures used have been described in detail elsewhere (Rosenthal and Danielsson, 1978). PPD tuberculin was obtained from Statens Seruminstitut, Copenhagen, as a solution of 1 mg/ml in phosphate buffer, without addition of preservative.

Mitogen

PHA, obtained from Wellcome Foundation, was used in a final dilution of 1:100.

STATISTICS

The mean values are given in log transformed counts/min $\pm$ SE. Student's *t* test was used to estimate statistical significance.

Results

The lymphocyte reactivity to the pathogenic (T₂) and non-pathogenic *Neisseria* (NPN) antigens was studied in 42 patients with uncomplicated and urogenitally complicated gonococcal urethritis and in 18 controls. The results are summarised in Table 1. The lymphocyte response showed no significant differences between patients and controls or between T₂-antigen stimulated and NPN-antigen stimulated cell cultures. The lymphocyte reactivity was therefore analysed in accordance with the following criteria: (a) number of gonococcal infections, (b) presence of urogenital complications, (c) serological response in the GCFT, and (d) sex distribution; this lymphocyte reactivity was compared with that in the controls. The patients with gonococcal urethritis were subdivided into three groups: single infection, multiple infection, and urogenital complications in the form of prostatitis and salpingitis (Table 2). The thymidine uptake in stimulated cell cultures was nearly the same in all the groups of patients. There was, however, a significant difference in the gonococcal-antigen-stimulated DNA synthesis between the 21 female

Table 1 *T₂-induced and NPN-induced DNA syntheses in cells from 42 patients with gonococcal urethritis and from 18 blood donor controls*

	10 log counts/min $\pm$ SE (antilogs within parentheses)	
	T ₂	NPN
Patients	3.757 $\pm$ 0.069 (5716)	3.885 $\pm$ 0.065 (7673)
Controls	3.622 $\pm$ 0.090 (4185)	3.933 $\pm$ 0.084 (8570)
<i>t</i> test between patients and controls	<i>t</i> = 1.12 (<i>P</i> > 0.05)	<i>t</i> = 0.42 (<i>P</i> > 0.05)

Table 2 Induction of DNA synthesis by T_2 and NPN antigens in lymphocytes from 42 patients with gonococcal urethritis and from 18 blood donor controls

Diagnosis	No. of patients	10 log counts/min $\pm$ SE (antilogs within parentheses)	
		T_2	NPN
Single infection	17	3.824 $\pm$ 0.073 (6669)	3.949 $\pm$ 0.092 (8892)
Multiple infections	10	3.579 $\pm$ 0.164 (3793)	3.729 $\pm$ 0.143 (5357)
Urogenital complications	15	3.800 $\pm$ 0.135 (6309)	3.916 $\pm$ 0.117 (8241)
Controls	18	3.622 $\pm$ 0.090 (4185)	3.933 $\pm$ 0.084 (8570)

patients and the 18 controls, t values being 2.40 $P < 0.05$ for log counts/min. No difference was observed between the 21 male patients and the controls (Table 3).

The GCFT was found to be positive in 12 of the 42 patients (Table 4). A study of the possible correlation between the outcome of the serological test and the results of the cellular reactivity to gonococcal antigen gave the following results (Figure). The ^{14}C -thymidine uptake in lymphocyte cultures from the 16 male patients with negative GCFT stimulated with T_2 antigen was much lower than the thymidine uptake in stimulated cell cultures from the other 26 male and female patients (t value=4.01 $P < 0.01$). The difference was significant between the five seropositive and the 16 seronegative men (t value=3.03 $P < 0.01$), but not between the seven seropositive and 14 seronegative women (t value=0.20). The DNA synthesis

Table 4 Presence of complement-fixing antibodies in different patient groups

Diagnosis	Male patients		Female patients	
	Positive GCFT	Negative GCFT	Positive GCFT	Negative GCFT
Single infection	2	4	6	5
Multiple infections	1	7	0	2
Urogenital complications	2	5	1	7
Total	5	16	7	14

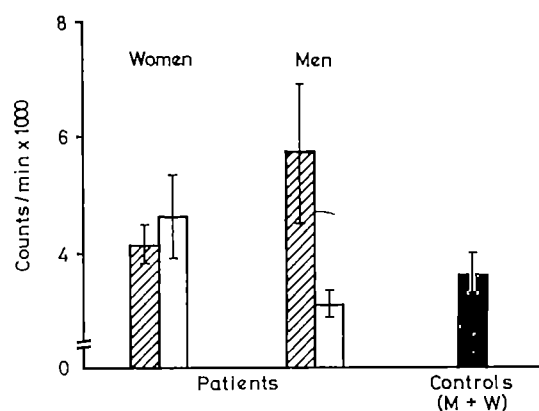


Figure Lymphocyte reactivity to T_2 in cell cultures from 21 female and 21 male patients with (hatched) and without (white) the presence of complement-fixing antibodies, and in cell cultures from blood donors (black). The bars represent the mean counts/min $\pm$ SE in patients and controls.

Table 3 Induction of DNA synthesis by T_2 in lymphocytes from male and female patients with gonorrhoea and from blood donor controls

Male patients				Female patients			
Diagnosis	No. of patients	10 log counts/min $\pm$ SE (antilogs within parentheses)	t values	Diagnosis	No. of patients	10 log counts/min $\pm$ SE (antilogs within parentheses)	t values
Single infection	6	3.628 $\pm$ 0.126 (4246)	0.04	Single infection	11	3.931 $\pm$ 0.075 (8531)	2.38*
Multiple infections	8	3.553 $\pm$ 0.206 (3572)	0.37				
Prostatitis	7	3.630 $\pm$ 0.178 (4265)	0.05	Salpingitis	8	3.949 $\pm$ 0.194 (8892)	1.76
Total patients	21	3.595 $\pm$ 0.113 (3935)	0.16	Total patients	21	3.914 $\pm$ 0.082 (8203)	2.40*
Controls (male and female)	18	3.622 $\pm$ 0.090 (4187)		Controls (male and female)	18	3.622 $\pm$ 0.090 (4187)	

t = statistical significance between the respective patient group and the control group

* $P < 0.05$

was higher in cell cultures from seronegative women than from seronegative men (t value = 3.10 $P < 0.01$). A significant difference (t value = 2.76 $P < 0.01$) was also noted in the lymphocyte reactivity to gonococcal-antigen between the controls and all the patients except those men who gave negative results in serological tests. There was no difference between these two groups with respect to the thymidine uptake in NPN-stimulated cell cultures (t value = 0.78). The lymphocyte response to PHA and PPD was not significantly different in any of the above-mentioned groups and subgroups. There was no difference in DNA synthesis between the T_4 stimulated cell cultures from male and female controls.

Discussion

In the present study there were no demonstrable differences in the lymphocyte response to gonococcal antigen between the 42 patients with gonococcal urethritis and the 18 blood donor controls (Table 1). A comparison of this lymphocyte reactivity between the controls and each of the subdivided groups showed the same results. These findings confirm previous observations by Rosenthal (1972, 1975). One explanation of these results could be the presence of asymptomatic male and female carriers of gonococcal infection in the group of controls (Carpenter and Westphal, 1940; Pariser, 1972; Handsfield *et al.*, 1974). In previous studies on the lymphocyte response to gonococcal antigen in patients with chronic Reiter's disease, Rosenthal (1972, 1973, 1976) observed that 41% of the lymphocytes from controls gave an intensified cellular reaction in response to gonococcal antigen. Gonococci have many antigens in common with meningococci. Rosenthal and Danielsson (1978) found a close antigenic relationship between these microorganisms. Therefore the possibility of an immune response developing as a result of meningococcal infection cannot be excluded.

A significant difference (t values = 2.40 $P < 0.05$) in lymphocyte reactivity was noted only between female patients and controls (Table 3). It has been postulated that a persistent exposure to the gonococci results in a more intense antigenic stimulation (Hess *et al.*, 1965; Kraus *et al.*, 1970, 1975) which could be one of the reasons for a humoral and cellular immune response. With the lymphocyte-stimulation test, as with the serological test (Ratnatunga, 1971), a stronger response to gonococcal antigens is observed in women than in men. This could be because a larger area of mucous membrane is exposed to the infec-

tion. The duration and dose of infection may be greater in women because of the delay in diagnosis owing to lack of symptoms or to the presence of latent complications, for instance salpingitis. The duration of symptoms before diagnosis and sampling was short in all the six male patients with single gonococcal urethritis but only in two of 13 females with uncomplicated urethritis. In the male patients with repeated gonococcal urethritis the history of the present infection was also short (between one day and three weeks). It should be pointed out that except for two patients the others had had only one previous infection.

Surprisingly few sera from the group of patients with urogenital complications and from those with multiple infections gave positive results in the GCFT in our study, which contrasts with previous experience (Danielsson *et al.*, 1972). The sera from patients with prostatitis and salpingitis were obtained two years after the collection of the strains for antigen production, while the sera from patients with uncomplicated gonococcal urethritis were collected at the time of antigen production. The results of the serological analyses were obtained with the same antigen preparation comprising five strains. The lack of reactivity could be due to a change in the population of strains that cause infection or to the absence of antigenic determinants in the strains chosen for antigen production. Unfortunately, the gonococcal strain used to produce antigen for the lymphocyte-stimulation test was not available for the GCFT. In the absence of methods to select strains for antigen production, one has to rely either on single strains that have been proved to possess a broad antigenic spectrum (Torrey, 1940; Welch and O'Reilly, 1973) or on a collection of strains thought to be typical of the spectrum of strains present in the population under study. The importance of careful selection of strains is stressed by the finding that even in neighbouring areas there exist significant differences in the antigens to which the population is exposed (Sandström and Danielsson, 1977). In the present study we were able to demonstrate the presence of a lymphocyte response to gonococcal antigen in some but not all of the patients with gonococcal infection. Gonococcal antigen can be present in prostatic secretion three weeks after infection in 20–40% of patients with clinically healed gonococcal urethritis (Danielsson and Molin, 1971).

The lymphocyte proliferation test might be a new diagnostic parameter in patients with chronic and latent gonococcal infection, as humoral antibodies cannot always be demonstrated in these patients.

This work was supported by grants from King Gustaf V's 80-årsfond and the Swedish Association against Rheumatic Disease. The authors thank Lola Markling and Birgitta Hagström for excellent technical assistance.

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Genital inoculation of male *Macaca fascicularis* with *Neisseria gonorrhoeae* and *Ureaplasma urealyticum*

WILLIAM R. BOWIE*, RONALD F. DIGIACOMO, KING K. HOLMES AND JAMES L. GALE

From the Department of Medicine, US Public Health Service Hospital; the Department of Epidemiology, School of Public Health and Community Medicine; and the Regional Primate Research Center, University of Washington, Seattle, Washington

SUMMARY Inoculation of the urethra, conjunctiva, pharynx, and anal canal of six male crab-eating macaques (*Macaca fascicularis*) with urethral exudate from male patients with urethral gonorrhoea or with laboratory gonococcal strains was unsuccessful in establishing infection or producing increased polymorphonuclear leucocytes on Gram stain. Intraurethral inoculation with laboratory strains of *Ureaplasma urealyticum* resulted in transiently positive urethral cultures for *U. urealyticum* and was associated in some cases with increased numbers of polymorphonuclear leucocytes on Gram stain of urethral material. The findings suggest that the crab-eating macaque may be useful for studying the pathogenesis of urethral infection with *U. urealyticum*.

Introduction

Investigations of gonococcal and nongonococcal urethritis have been hampered by the lack of animal models in which urethral infection can be produced. Among nonhuman primates only chimpanzees (*Pan troglodytes*) have been convincingly infected with *Neisseria gonorrhoeae* (Lucas *et al.*, 1971); other simians have proved resistant to urethral infection (DiGiacomo *et al.*, 1977; Gale *et al.*, 1977). Urethral infection with *Chlamydia trachomatis* has been produced in baboons (*Papio cynocephalus*) (DiGiacomo *et al.*, 1975) and pig-tailed macaques (*Macaca nemestrina*) but not in capuchins (*Cebus albifrons*) (Gale *et al.*, 1977). *Ureaplasma urealyticum* urethral infection has been produced in one of two pig-tailed macaques but not in baboons or capuchins (Gale *et al.*, 1977). Experiments with other simians may reveal a more suitable nonhuman primate model for studying the pathogenesis and immunity of urethral infection

with *N. gonorrhoeae*, *C. trachomatis*, and *U. urealyticum*. Furthermore, whereas *N. gonorrhoeae* and *C. trachomatis* are recognised causes of urethral disease, the role of *U. urealyticum* in urethral disease remains equivocal. Therefore, a previously untested simian species, the crab-eating macaque (*Macaca fascicularis*), was inoculated with *N. gonorrhoeae* and *U. urealyticum*.

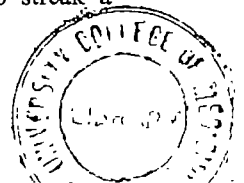
Materials and methods

Six adult male *Macaca fascicularis* weighing between 3 and 8.5 kg were used. Monkeys were individually caged and fed commercially prepared monkey pellets. Before inoculation and specimen collection the monkeys were sedated with ketamine hydrochloride (Vetalar, Parke-Davis and Co., Detroit, Michigan). Specimens were obtained from the urethra and conjunctiva with calcium alginate urethrogenital swabs, and from the pharynx and anal canal with cotton swabs. Urethral cultures were obtained by serially advancing swabs more proximally. The first swab was used to prepare a Gram stain and it was then placed in transport medium (0.2 mol/l sucrose in 0.02 mol/l phosphate buffer, pH 7.0) for *C. trachomatis* isolation and frozen at -70°C. The second swab was used to streak a

*Currently at the University of British Columbia, Faculty of Medicine, Vancouver General Hospital, Vancouver, British Columbia, Canada, V5Z 1M9

Address for reprints: W. R. Bowie, Faculty of Medicine, Vancouver General Hospital, Vancouver, British Columbia, Canada V5Z 1M9

Received for publication 12 December 1977



Thayer-Martin culture plate for *N. gonorrhoeae* isolation. The third swab was used to streak an A6 plate (Shepard and Lunceford, 1970) for *Mycoplasma hominis* and *U. urealyticum* isolation and then it was placed in broth (Holmes *et al.*, 1975) for *U. urealyticum* isolation. A similar procedure was used in the conjunctiva, pharynx, and anal canal except that Gram stain preparations were not made. *C. trachomatis*, *N. gonorrhoeae*, and *U. urealyticum*, were isolated and identified as previously described (Holmes *et al.*, 1975).

Urethral specimens from men with urethral discharge were obtained and cultured for *N. gonorrhoeae*, *C. trachomatis*, *U. urealyticum*, and *M. hominis* (Holmes *et al.*, 1975). Fresh urethral exudate from five men with gonococcal urethritis, diagnosed by Gram stain and later confirmed by culture, was used to inoculate five of the six monkeys. Patients were taken to the primate centre, urethral exudate was obtained on swabs and immediately inoculated into the monkeys. One swab was used to inoculate the urethra and conjunctiva, and a second swab was used to inoculate the pharynx and anal canal of the monkeys. Urethral exudate from one patient was used to inoculate one monkey. Inoculated monkeys were examined, and cultures were taken for *N. gonorrhoeae*, *C. trachomatis*, *U. urealyticum*, and *M. hominis* between three and five, seven, 14, and 28 days after inoculation. The uninoculated monkey was examined and cultures were taken 28 days after the initial study.

Three months after inoculation of urethral exudate from patients, the six monkeys were inoculated with two laboratory strains of *N. gonorrhoeae* that had been passaged approximately 10 times as type 2 colonies. One strain, typical of isolates from men with asymptomatic gonorrhoea, required arginine, hypoxanthine, and uracil for growth, while the other strain, typical of isolates from men with symptomatic gonorrhoea, did not require arginine, hypoxanthine, and uracil for growth (Crawford *et al.*, 1977). Gonococci were streaked on to GC agar base with Kellogg's supplement (Baltimore Biological Laboratories, Baltimore, Maryland) and incubated for 16 hours in a candle extinction jar at 36.5°C. Organisms were suspended in trypticase soy broth at a concentration of 10⁷ colony-forming units per ml as determined by dilution plate counts on GC agar. A catheter was used to inoculate 0.5 ml of broth into the urethra while inoculum-soaked swabs were used to inoculate the conjunctiva, pharynx, and anal canal. Each strain was used to inoculate three monkeys. The six monkeys were examined and cultured two and seven days after inoculation.

Two strains of *U. urealyticum* isolated from the urethra of two men with acute epididymitis associated with nongonococcal urethritis were used to inoculate the monkeys three weeks after inoculation of the *N. gonorrhoeae* cultures. The strains were isolated in broth, and at the onset of colour change denoting a rise in pH, the cultures were frozen at -70°C. The cultures were then thawed, passed into new broth, and incubated. To ensure that the inoculum contained only *U. urealyticum*, the organisms were terminally diluted three times. The highest dilution showing colour change in the third passage was subcultured into broth. A 14-hour growth of this subculture was used as the inoculum. It contained 10⁶ colour changing units (CCU) of *U. urealyticum* per ml. A catheter was used to inoculate 0.5 ml of inoculum into the urethra, and an inoculum-soaked swab was used to inoculate the conjunctiva. Each strain was used to inoculate three monkeys. Monkeys were examined, and the urethra, conjunctiva, and pharynx were cultured one, two, three, six, and eight weeks after inoculation. Five months after the primary inoculation with the *in vitro* cultures of *U. urealyticum*, all six monkeys were reinoculated with the same strains of *U. urealyticum*. This inoculum contained 10⁷ CCU per ml and 0.5 ml was again inoculated into the urethra. Monkeys were examined and the urethra cultured three, seven, 10, 14, 21, and 28 days after inoculation.

Results

BASELINE STUDIES OF MONKEYS

All specimens obtained between one and five weeks earlier and immediately before inoculation with urethral exudate were negative for *N. gonorrhoeae*, *C. trachomatis*, and *U. urealyticum*. Polymorphonuclear leucocytes (PMN) were not seen on Gram-stained smears of baseline urethral swabs (Table). All pharyngeal specimens and one anal canal specimen yielded typical mycoplasma colonies which appeared urease-positive on A6 agar. The specimens which yielded these colonies also partially changed the colour of the phenol red pH indicator in urea broth towards alkaline. Specimens which did not yield these colonies did not alter the pH. However, neither the organisms grown on agar nor those changing the pH in broth could be passed in either agar or broth. The identity of these organisms was not determined, but the organisms were frequently isolated from the pharynx, and occasionally from the anal canal throughout the study.

Table Urethral inoculation of *Macaca fascicularis* with *Ureaplasma urealyticum*

Monkey no.	Pre-inoculation PMN*	Urethral exudate inoculated			U. urealyticum inoculated			U. urealyticum reinoculated		
		U. urealyticum			U. urealyticum			U. urealyticum		
		Inoculum†	Isolated at follow-up‡	PMN*	Inoculum†	Isolated at follow-up‡	PMN*	Inoculum†	Isolated at follow-up‡	PMN*
6870	0	10 ⁴	No	0-2	10 ⁴	No	3-5	10 ⁷	3, 7	0-1
7088	0	10 ⁴	No	0	10 ⁴	7, 14, 21, 42	3-5	10 ⁷	No	0-1
70564	0	—	—	—	10 ⁴	7	0-1	10 ⁷	10	12-18
75231	0	10 ¹	No	0-2	10 ⁴	Contaminated	3-5	10 ⁷	7, 10	3-4
75237	0	10 ¹	No	0	10 ⁴	7	3-4	10 ⁷	3, 7	3-6
75242	0	0	No	0	10 ⁴	Contaminated	0-1	10 ⁷	7	0

*Range of the number of polymorphonuclear leucocytes per $\times 1000$ oil immersion field on Gram stain of urethral smears

†Concentration of inoculum expressed as the number of colour changing units per ml of first-voided urine in the inoculation of urethral exudate, and per ml of urea broth for the *U. urealyticum* inoculations

‡Days after inoculation at which *U. urealyticum* was isolated

Inoculation with urethral exudate from men with urethral gonorrhoea

The presence of *N. gonorrhoeae* in patients was confirmed by growth on Thayer-Martin medium of oxidase-positive Gram-negative diplococci which fermented glucose, but not maltose, sucrose, or lactose. In addition, four patients had 10¹ to 10⁴ CCU of *U. urealyticum* per ml of first-voided urine, but it is not known how many *U. urealyticum* organisms were present in the urethral exudate that was inoculated. The results of follow up cultures for *U. urealyticum* are shown in the Table. Two patients also had *C. trachomatis* isolated from the urethral exudate. Monkeys (nos 7088 and 75231) received exudate from these men. However, all follow-up specimens for the inoculated sites in monkeys were negative for *N. gonorrhoeae*, *C. trachomatis*, and *U. urealyticum*. No discharge was observed from any site, and only an occasional PMN per $\times 1000$ oil immersion field was observed on examination of the entire Gram stain.

Inoculation with in vitro cultures of *N. gonorrhoeae*

Specimens from monkeys inoculated with laboratory cultivated gonococci were negative except for one monkey, from which *N. gonorrhoeae* was obtained from the pharynx two days after inoculation. Gram-negative extracellular diplococci were also observed on the urethral smear from this monkey but viable gonococci were not isolated from the urethra. All specimens collected seven days after inoculation gave negative results. No discharge from the urethra or conjunctiva, or inflammation of the conjunctiva, pharynx, or anal canal was observed. Zero to 1 PMN per $\times 1000$ field was observed in urethral smears.

Inoculation with in vitro cultures of *U. urealyticum*

The results are shown in the Table. One week after inoculation, the organism was recovered from the

urethra of three monkeys and was not isolated from one. Cultures made from the remaining two monkeys were contaminated with bacteria. Urethral specimens collected two weeks after inoculation were negative except for one monkey in which infection continued for six weeks. *U. urealyticum* was not isolated from the conjunctiva. There was no evidence of a urethral discharge, but four monkeys exhibited 3-5 PMN per $\times 1000$ field on the urethral smear.

Reinoculation with in vitro culture of *U. urealyticum*

Before reinoculation five months after the initial inoculation of *U. urealyticum*, urethral cultures were negative for *U. urealyticum* and no PMN were observed on urethral smears. In five of the six monkeys *U. urealyticum* was reisolated for seven to 10 days after reinoculation (Table), but subsequent cultures were negative. The monkey that had previously shed *U. urealyticum* for six weeks was not reinfected and did not have an increased number of PMN on Gram stain. No urethral discharge was present in the monkeys, but two had 3-6 and one had 12-18 PMN per $\times 1000$ field on the urethral smear.

Discussion

No infection of the urethra, conjunctiva, pharynx, and anal canal of *Macaca fascicularis* was produced by inoculating urethral exudate from men with gonorrhoea, or of *in vitro* cultures of *N. gonorrhoeae*. However, inoculation of *in vitro* cultures of *U. urealyticum* initially isolated from men with epididymitis, resulted in positive cultures in three of four monkeys with uncontaminated cultures after primary inoculation, and in five of six monkeys after reinoculation. Cultures were only transiently positive in all but one monkey. This does not necessarily indicate that the monkeys were infected,

but infection is possible because in some of the animals an increased number of PMN was seen on urethral Gram stain concurrent with isolation of *U. urealyticum*. More than 3 PMN per $\times 1000$ field on urethral Gram stain were not seen in the baseline studies, after the inoculations with urethral exudate and *N. gonorrhoeae*, nor after inoculations with *U. urealyticum* at times when cultures for *U. urealyticum* were negative. Except for one monkey, 3 or more PMN per $\times 1000$ field were detected only at times when cultures for *U. urealyticum* were positive. In men attending a sexually transmitted diseases clinic a mean of over 4 PMN per $\times 1000$ field on urethral Gram stain, obtained when the patient had not voided for two hours or longer, correlated with increased numbers of PMN in the sediment of the first-voided urine (Bowie, 1978). The number of PMN seen in the monkeys' urethral exudate is probably significant when it is considered that the monkeys invariably voided during the sedation procedure.

As there were only six monkeys, none was given a control inoculation with urea broth without *U. urealyticum*. It is unlikely that the physical trauma of inoculating the urethra or obtaining repeated urethral cultures produced the increase in numbers of PMN because they did not occur with the inoculations of *N. gonorrhoeae* and the PMN disappeared although further follow-up cultures were obtained. However, the possibility that some component of the urea broth medium elicited the increase in numbers of PMN has not been ruled out.

These studies support clinical studies which implicate *U. urealyticum* as a urethral pathogen in humans. In men with nongonococcal urethritis experiencing their first episode of urethritis, the isolation rate of *U. urealyticum* was significantly higher from *C. trachomatis* negative patients than from *C. trachomatis* positive patients or from men without urethritis who were comparable in sexual history (Bowie *et al.*, 1977). Furthermore, treatment of *C. trachomatis*-negative, *U. urealyticum*-positive urethritis with aminocyclitols resulted in improvement of the urethritis when *U. urealyticum* was eradicated, but not when it persisted (Bowie *et al.*, 1976). Experimental inoculation of the human urethra with *U. urealyticum* has been performed in two men (Taylor-Robinson *et al.*, 1977). Both men developed threads in the urine, and one developed a mucoid discharge.

There are problems with using *Macaca fascicularis* as a model. The animals invariably micturate during the sedation procedure, and if a urethral discharge is produced it would probably be washed

away. Micturition decreases the number of PMN that can be detected on urethral smear and may have interfered with detection of low concentrations of *U. urealyticum*. Because of the small size of the urethra in this species, urethroscopy cannot be performed. Nevertheless, this is the second non-human primate in which urethral *U. urealyticum* infection has been produced, and nonhuman primates may possibly serve as an alternative to humans. Such models could be used to explore the relative pathogenicity of various types of *U. urealyticum*, and perhaps to analyse the steps in the pathogenesis of urethritis and the evolution of specific or heterologous immunity.

This study was supported by Public Health Service Grants AI-12192 from the National Institute of Allergy and Infectious Diseases and RR-00166 from the Division of Research Resources, and a Medical Research Council of Canada Fellowship (WRB).

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Effects of various divalent cations on the survival of *Neisseria gonorrhoeae* in liquid media

TOLU ODUGBEMI, MICHAEL McENTEGART, AND SALEEM HAFIZ

From the Department of Medical Microbiology, University of Sheffield Medical School, Sheffield

SUMMARY The effect of various concentrations of divalent cations on survival of gonococci in liquid medium was studied. The growth of *Neisseria gonorrhoeae* was inhibited by manganous chloride in concentrations above 1×10^{-6} mol/l while the growth of control organisms such as *Neisseria meningitidis* and *Escherichia coli* was not inhibited by the same salt even at 1×10^{-2} mol/l. Copper sulphate, cobaltous nitrate, nickel chloride, and zinc sulphate also had deleterious effects on gonococci. Magnesium chloride at 1.5×10^{-1} mol/l permitted the growth of gonococci. The toxicity of manganous chloride and copper sulphate in the liquid media was in some measure reduced by adding charcoal but not by adding starch. The significance of these findings is discussed in relation to the efficiency of primary isolation and transport media for gonococci.

Introduction

Müller and Hinton (1941) first reported satisfactory primary growth of gonococci on a solid medium free of animal protein but incorporating starch which, they suggested, acted as a 'protective colloid' against the inhibiting effects of certain amino-acids. Glass and Kennett (1939) had already shown that particulate carbon had a protective effect on gonococci in liquid media. In a study aimed at identifying inhibitory substances present in commercial agars Ley and Müller (1946) identified a substance which was similar to a fatty acid.

Recently, Miller *et al.* (1977) studied the inhibitory action on the growth of *Neisseria gonorrhoeae* of the fatty acids commonly present in media and showed that such inhibition of growth increased with increasing chain length of the saturated fatty acids up to a maximum with palmitic acid (C16). Unsaturated fatty acids with chain lengths of C16 to C20 were also found to be inhibitory.

If the development of media for the study of gonococci is to be other than empirical, it is essential to increase our understanding of the effect various components have on the growth of gonococci. Bridson and Brecker (1970) found various concentrations of the divalent cations manganese (Mn^{++}), copper (Cu^{++}), nickel (Ni^{++}), and cobalt (Co^{++}) in

peptones and agar; it therefore seemed a useful starting point to study the effect of these substances on gonococci. We therefore incorporated concentrations of manganous chloride, copper sulphate, cobaltous nitrate, nickel chloride, zinc sulphate, and magnesium sulphate into ANM media (Hafiz and McEntegart, 1976) and studied their effect on the survival of gonococci.

Materials and methods

MEDIA

The studies were carried out in liquid medium, ANM, which was prepared as follows: proteose peptone (Difco 3) 15 g, sodium chloride (Analar) 5 g, dipotassium phosphate (Analar) 4 g, potassium di-hydrogen phosphate (Analar) 1 g, soluble starch (Analar) 1 g, sodium bicarbonate (Analar) 0.15 g, glucose (Analar) 5 g, and distilled water 1 litre.

This basic liquid medium was adjusted to pH 7.2 with N/NaOH (the endpoint being read by means of an EIL laboratory pH meter no. 7020) and sterilised by autoclaving at 110°C for 10 min. Solutions were sterilised by filtering through a standard Seitz filter using EK/5 (sterilising grade) pads. Filtration was preferred to autoclaving which might have caused precipitation. Solutions of the following salts were processed and added to the liquid media to give the following final concentrations:

Address for reprints: T. Odugbemi, Department of Medical Microbiology, University of Sheffield Medical School, Beech Hill Road, Sheffield S10 2RX

Received for publication 24 February 1978

Manganous chloride ($\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$) 1×10^{-3} mol/l to 2×10^{-3} mol/l

Cupric sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) 4×10^{-4} mol/l to 4×10^{-3} mol/l

Cobaltous nitrate $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ 2×10^{-3} mol/l to 5×10^{-3} mol/l

Nickel chloride ($\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$) 2×10^{-3} mol/l to 2×10^{-4} mol/l

Magnesium chloride ($\text{MgCl}_2 \cdot 7\text{H}_2\text{O}$) 1.5×10^{-1} mol/l to 1×10^{-3} mol/l.

All the salts were analytical reagent grade from BDH Laboratories. The media containing the various cations were distributed in 10 ml volumes in universal containers.

STRAINS

The following strains of *N. gonorrhoeae* were used in the study: F62 (originally isolated in Kellogg's laboratory and stored in liquid nitrogen in our laboratory), strains designated GC41, GC338, GCR46, 77/6182, and AN524, all freshly isolated from patients attending the Special Clinic, Royal Infirmary, Sheffield, and the R1 β -lactamase producing strain (kindly provided by Dr A. E. Jephcott). The identity of the gonococci was confirmed by Gram staining, oxidase reaction, sugar fermentation patterns, and bright fluorescence with specific antigenococcal fluorescein conjugate (Difco). The following bacteria were used as controls: *Neisseria meningitidis*, *Branhamella* (*Neisseria*) *catarrhalis*, *Neisseria pharyngis*, and *Escherichia coli*.

A suspension of each strain was made from a culture on Difco GC medium in phosphate buffered saline pH 7.4 (Oxoid) and mixed thoroughly in a Junior mixer (Scientific Co. Ltd). The suspensions were standardised so as to contain approximately 10^8 colony forming units/ml, and 0.04 ml was inoculated into 10 ml volumes of the appropriate liquid medium.

After establishing the inhibitory action of certain cations in a subsequent experiment, the effects of activated charcoal (BDH) and soluble starch on the inhibition of gonococci by copper sulphate and manganous chloride were studied. Liquid media with the following concentrations of copper sulphate: $400 \mu\text{mol/l}$, $300 \mu\text{mol/l}$, $200 \mu\text{mol/l}$, and $100 \mu\text{mol/l}$ with or without charcoal 1% (mass/vol.) and starch 1% (mass/vol.) were inoculated with *N. gonorrhoeae* strain F62 as previously described. In the same way media containing manganous chloride at the following concentrations: $100 \mu\text{mol/l}$, $50 \mu\text{mol/l}$, $40 \mu\text{mol/l}$, $20 \mu\text{mol/l}$ and $10 \mu\text{mol/l}$ with or without charcoal or starch were inoculated with *N. gonorrhoeae* strains F62 and R1.

Cultures in liquid media and on solid GC media were all incubated at 35°C in an atmosphere of air with 10% carbon dioxide and enhanced humidity.

The growth and survival of gonococci were monitored by the daily subinoculation of the liquid cultures on to solid Difco GC medium plus 2% defined supplement (Kellogg *et al.*, 1963) using a standard bacteriological loop 4 mm diameter. Inoculated plates showing no growth by naked eye were examined under the stereomicroscope ($\times 40$ magnification), and when in doubt about the identity of any growth the Gram staining, oxidase test, and sugar fermentation reactions were checked.

Results

All the strains of gonococci tested, including 100 strains freshly subcultured from storage in liquid nitrogen, were inhibited by manganous chloride at a concentration of 1×10^{-3} mol/l, which had no effect whatsoever on the growth of the control organisms *N. pharyngis*, *N. meningitidis*, and *E. coli*. When lower concentrations of manganous chloride were tested it was found that the highest concentration that permitted the growth of gonococci was about 1×10^{-4} mol/l. The effects of the various concentrations of the divalent cations incorporated into the liquid media (ANM) on the survival of gonococci are shown in Table 1.

The maximum concentrations of the salts that permitted the growth of gonococci in the media were as follows: copper sulphate 2×10^{-4} mol/l, cobaltous nitrate 2.5×10^{-4} mol/l, nickel chloride 5×10^{-4} mol/l, and zinc sulphate 1×10^{-3} mol/l. The toxic effect of zinc sulphate on gonococci was different from that of the other salts tested. While other salts either permitted or prevented growth of a typical colonial form of gonococci the effect of zinc sulphate was more difficult to interpret, because even when growth occurred it was atypical. Very tiny colonies were seen under the stereomicroscope ($\times 40$ magnification) but these invariably failed to grow when subinoculated on to fresh medium. Magnesium chloride was not toxic to any of the strains tested at a concentration of 1.5×10^{-1} mol/l.

Table 2 shows the effects of charcoal and starch on the inhibition of gonococci by manganous chloride. It is interesting to note that in liquid medium concentrations of manganous chloride greater than $10 \mu\text{mol/l}$ inhibited growth of gonococcal strains F62 and R1, whereas in the presence of charcoal growth occurred in concentrations up to $40 \mu\text{mol/l}$; thus it appeared that in the presence of charcoal the organism could tolerate four times the normal inhibitory concentration.

Table 1 Effects of various concentrations of divalent cations incorporated into liquid media on the survival of gonococci

	Salts															
	MnCl ₂ ·4H ₂ O		CuSO ₄ ·5H ₂ O		Co(NO ₂) ₆ ·6H ₂ O		NiCl ₂ ·6H ₂ O		ZnSO ₄ ·7H ₂ O		MgCl ₂ ·7H ₂ O					
Concentration of salts in liquid media (mol/l)	1 × 10 ⁻² m	1 × 10 ⁻³ m	4 × 10 ⁻⁴ m	2 × 10 ⁻⁴ m	1 × 10 ⁻⁴ m	2 × 10 ⁻³ m	1 × 10 ⁻² m	5 × 10 ⁻⁴ m	2.5 × 10 ⁻⁴ m	2 × 10 ⁻³ m	1.5 × 10 ⁻² m	5 × 10 ⁻⁴ m	4 × 10 ⁻³ m	2 × 10 ⁻³ m	1 × 10 ⁻² m	1.5 × 10 ⁻¹
<i>N. gonorrhoeae</i>																
F62	—	+	—	—	+	—	—	—	+	—	—	+	—	—	+	+
R1	—	+	—	—	+	—	—	—	+	—	—	+	—	A	A	+
41	—	+	—	—	+	—	—	—	+	—	—	+	—	A	A	+
R46	—	+	—	—	+	—	—	—	+	—	—	+	—	A	A	+
77/6182	—	+	—	—	+	—	—	—	+	—	—	+	—	A	A	+
338	—	+	—	—	+	—	—	—	+	—	—	+	—	A	A	+
AN524	—	+	—	—	+	—	—	—	+	—	—	+	—	A	A	+
<i>N. meningitidis</i>	+	+	—	+	+	—	—	+	—	—	—	+	—	A	A	+
<i>B. catarrhalis</i>	+	+	+	+	+	—	+	+	+	—	+	+	A	+	+	+
<i>N. pharyngis</i>	+	+	+	+	+	—	+	+	+	—	+	+	A	+	+	+
<i>E. coli</i>	+	+	+	+	+	—	+	+	+	+	+	+	A	+	+	+

— No growth

+ Growth

A Very tiny atypical growth

Table 2 Effects of charcoal and starch on the manganous chloride inhibition of gonococci

Culture medium	Strains	Concentrations of MnCl ₂ ·4H ₂ O in liquid medium				
		100 µmol/l	50 µmol/l	40 µmol/l	20 µmol/l	10 µmol/l
MnCl ₂ ·4H ₂ O only	F62	—	—	—	—	+
	R1	—	—	—	—	+
plus charcoal	F62	—	—	+	+	+
	R1	—	—	+	+	+
plus starch	F62	—	—	—	+	+
	R1	—	—	—	—	+

— No growth

+ Growth

The addition of starch to the media showed little ability to reverse the inhibition of gonococci by manganous chloride. (This effect is not unexpected as starch 0.1% (mass/vol.) is a normal constituent of the liquid media.) Charcoal also successfully reversed the inhibition of gonococci by copper sulphate (Table 3). Thus concentrations of copper sulphate greater than 100 µmol/l inhibited the growth of gonococci F62, whereas in the presence of charcoal growth occurred at concentrations of copper sulphate up to 400 µmol/l. Again starch was without effect in reversing this inhibition.

Table 3 Effects of charcoal and starch on the copper sulphate inhibition of *N. gonorrhoeae* (Strain F62)

Culture medium	Concentration of CuSO ₄ ·5H ₂ O in liquid media			
	400 µmol/l	300 µmol/l	200 µmol/l	100 µmol/l
CuSO ₄ ·5H ₂ O	—	—	—	+
plus charcoal	+	+	+	+
plus starch	—	—	—	+

— No growth

+ Growth

Discussion

Previous studies (Fiscina *et al.*, 1973; Cohen and Thomas 1974; Elmros *et al.*, 1976; Johannisson *et al.*, 1976) demonstrated the toxic effects of copper on gonococci. The present study attempts to quantitate the inhibitory action of various concentrations of copper sulphate and other divalent salts that may be present in various amounts in commercial peptones and agar.

The toxic effect of copper on gonococci is confirmed by the finding that concentrations above 2×10^{-4} mol/l would inhibit the growth of gonococci in the liquid media. It was interesting to find that manganous chloride had a specific inhibitory effect on gonococci at concentrations above 1×10^{-3} mol/l whereas the control organisms, *N. meningitidis*, *B. catarrhalis*, *N. pharyngis*, and *E. coli* grew in the liquid media up to concentrations of manganous chloride 1×10^{-3} mol/l. Cobaltous nitrate, nickel chloride, and zinc sulphate were also able to inhibit the growth of gonococci but not the control organisms.

The cumulative effects of these various toxic salts in different commercial agars and peptones could in part account for the poor growth of gonococci on these media.

It has been known for some time that fatty acids and amino-acids are toxic to gonococci, and the removal of these inhibitory factors may explain the beneficial effect of the addition of enrichment materials such as blood, starch, and charcoal to culture and transport media (Gerhardt and Heden, 1960).

The addition of charcoal to our liquid culture medium effectively reduced by about fourfold the toxic effects of manganous chloride and copper sulphate. The addition of starch to the liquid media had very little effect in reducing the toxic action of copper or manganese.

The efficiency of the liquid media designed for the primary isolation or transportation of gonococci may further be enhanced by the incorporation of about 0.5% (mass/vol.) charcoal.

A better understanding of the nature of the various toxic materials which may be present in culture media should make possible a rational improvement in the media used for the study of *N. gonorrhoeae*.

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Talampicillin and probenecid compared with ampicillin and probenecid for the treatment of gonococcal urethritis in men

S. AL-EGAILY,* E. M. C. DUNLOP,* P. RODIN,* AND A. D. SETH†

From the *Whitechapel Clinic, and the †Venereal Diseases Reference Laboratory, The London Hospital, London E1

SUMMARY Two hundred and ten men suffering from uncomplicated gonococcal urethritis were treated with one of two treatment schedules. Of 109 patients treated with 3 g ampicillin and 2 g probenecid (group A) there were two recurrences in the first week after treatment and none in the second week. Of 101 patients treated with 1.5 g talampicillin and 2 g probenecid (group B), there were three recurrences in the first week and none in the second week. Both antibiotics were well tolerated, but one patient vomited two hours after taking talampicillin. The sensitivity pattern of *Neisseria gonorrhoeae* to penicillin at The London Hospital has shown an increase in the proportion of more sensitive strains during the last three years. Talampicillin given in a single dose with probenecid is satisfactory in the treatment of acute gonococcal urethritis but, apart from the smaller dosage, it does not offer any advantage over ampicillin with probenecid.

Introduction

Talampicillin is a thiazolidine carboxylic ester of ampicillin which releases ampicillin after hydrolysis in the gut mucosa to produce concentrations in the blood up to twice as high as those achieved with ampicillin. Urine levels are also greater. Its absorption is affected by food to a lesser extent, and it has less effect on the faecal flora than ampicillin (Leigh *et al.*, 1976).

Willcox (1976) treated 81 patients for gonococcal urethritis with a single oral dose of 1.5 g talampicillin, without probenecid, and reported a failure rate of only 4.2% among those seen at least once after treatment; it was without side effects. He concluded that talampicillin was the most potent ampicillin-like antibiotic so far available for the treatment of gonorrhoea and a smaller single dose was capable of curing the disease than would have been needed with ampicillin.

Price *et al.* (1977) treated 245 patients suffering from uncomplicated gonorrhoea with 1.48 g talampicillin and 1 g probenecid and reported a failure rate of only 1.6% in patients seen at least once after treatment.

Men presenting at The London Hospital with uncomplicated gonorrhoea are usually treated with a single oral dose of 3 g ampicillin and 2 g probenecid. It was decided to compare the result of this treatment (Group A) with that of talampicillin and probenecid (Group B) and to study the sensitivity pattern of *Neisseria gonorrhoeae* to penicillin to see if there had been any change since the report by Taylor and Seth (1975).

Men suffering from uncomplicated gonococcal urethritis were chosen for the trial.

As talampicillin gives peak serum ampicillin concentrations that are about twice as high as those with the same dose of ampicillin the results of treatment with 1.5 g talampicillin and 2 g probenecid (Group B) were compared with the results of treatment with 3 g ampicillin and 2 g probenecid (Group A).

Material and methods

Two hundred and ten men suffering from acute gonococcal urethritis were randomly allocated to one of two single-dose treatment schedules. Any patient who was allergic to penicillin, a habitual defaulter, unable to attend for regular follow-up, homosexual, or was suspected of having syphilis was excluded. Seventeen additional patients whose urethral smears contained classical Gram-negative,

Address for reprints: S. Al-Egaily, The Whitechapel Clinic, The London Hospital, Turner Street, London E1

Received for publication 21 February 1978

intracellular diplococci but in whom urethral cultures did not yield gonococci were not included in the analysis.

AGE AND NATIONALITY

The mean age, age range, and nationalities in the two groups are shown in Table 1.

FOLLOW-UP TESTS

The patients were advised to attend for follow-up on or about the 3rd, 7th, 14th, and 28th day after treatment. At each visit a smear and a culture were taken from the urethra whether or not there was obvious discharge.

Post-gonococcal urethritis was diagnosed from the eighth day onwards if the leucocyte count per high power field (HPF) with a total magnification of $\times 1000$ was over 30. These patients were treated with oxytetracycline 500 mg orally four times a day for two weeks.

DEFAULT RATE

Of the 210 patients in the trial 193 attended at least once after treatment (Table 2).

SENSITIVITY TESTS

Sensitivities to ampicillin and penicillin were determined by an agar plate dilution method and the minimum inhibitory concentrations (MIC) reported. Brain heart infusion agar (Oxoid) was

used for testing the sensitivity. Penicillin and ampicillin were incorporated in the following concentrations: 0.004, 0.008, 0.015, 0.03, 0.06, 0.125, 0.25, and 0.5 $\mu\text{g/ml}$. The inoculum consisted of a suspension of gonococci from a 24-hour-old plate subculture suspended in a nutrient broth (Oxoid) to give a turbidity approximately equal to a No. 2 MacFarland barium sulphate standard prepared in a nutrient broth. The plates containing the two antibiotics were inoculated by using a multipoint inoculator (Denley Instruments Ltd) the prongs delivering about 10^4 colony-forming units. The inoculated plates were incubated at 36°C in a candle extinction jar for 48 hours before readings were taken.

Staphylococcus aureus (Oxford) and two World Health Organisation *N. gonorrhoeae* reference strains III and V (A. Reyn, Statens Serum Institut, Copenhagen) were included as controls on each plate.

Results

Recurrences in the two groups are shown in Table 3.

GROUP A

There were eight recurrences. Of these six occurred after the second week of follow-up, and each patient admitted further sexual intercourse with the untreated contact or with a new partner later shown to be infected. It is likely that these six recurrences

Table 1 Age and nationality of patients in each group

Group	Age (years)		Nationality			Total
	Mean	Range	UK and Eire	Caribbean	Others	
A	27.8	16-47	35	45	29	109
B	30.2	18-60	48	36	17	101

Table 2 Duration of follow-up in each group

Group	Duration of follow-up (weeks)						Total
	0	1	2	3	4	>4	
A	14	95	84	56	44	31	109
B	3	98	82	59	45	37	101

Table 3 Results of treatment

Group	No. treated	No. followed up	Recurrences				
			First week		Second week	>2 weeks	
			No.	%		No.	%
A	109	95	2	2.1	0	6	6.3
B	101	98	3	3.1	0	10	10.1

were caused by reinfection. Two patients had recurrences in the first week. One patient admitted sexual intercourse with his untreated infected wife and had an organism sensitivity to 0.008 µg/ml ampicillin before and after treatment; this recurrence could well have been a reinfection. The second patient denied further sexual intercourse when he attended three days after treatment, and he was asymptomatic. Gonococci were not found in the smear, but were grown on culture, so his treatment was considered to have failed. The organism was sensitive to ampicillin 0.015 µg/ml. Both patients responded to kanamycin 2 g intramuscularly.

GROUP B

There were 13 recurrences. Of these 10 occurred after the second week, and the patients admitted further sexual intercourse with the untreated contact or with a new infected partner; these 10 recurrences were thus probably reinfections.

Of the remaining three recurrences, two were in patients who admitted further sexual intercourse with their untreated contacts. In one of these patients the organism was sensitive to 0.03 µg/ml ampicillin. In the other the organism was cultured three days after treatment and was sensitive to 0.03 µg/ml ampicillin. In the third patient, who vomited two hours after swallowing the tablets, the organism was sensitive to 0.25 µg/ml ampicillin. This patient denied further sexual intercourse when he attended three days after treatment. The organism isolated after treatment was sensitive to 0.5 µg/ml ampicillin. The recurrence in this patient was

considered as a failure of treatment; he responded to spectinomycin 2 g intramuscularly.

Post-gonococcal urethritis (PGU)

The incidence and time of detection are shown in Table 4. Most of the PGU was detected in the second week. The difference in incidence, 30% and 35%, was not significant ($\chi^2=1.094$; $P=0.3$).

Side effects

Apart from the patient who vomited two hours after swallowing the talampicillin and probenecid, no other side effects were observed.

RESULTS OF SENSITIVITY TESTS

The sensitivity of the gonococci to ampicillin and to penicillin was tested in 95 of the patients in group A and in 85 of those in group B (Tables 5 and 6).

The percentage of less sensitive strains to penicillin was a little higher in group A (Table 5), but the groups had virtually identical percentages of less sensitive strains to ampicillin (Table 6). The penicillin and ampicillin sensitivities of the strains of gonococci for those patients who subsequently had recurrences are shown in Table 7.

Discussion

Talampicillin with probenecid is an effective treatment for gonorrhoea, but in the dose used there was no significant difference in the outcome of treatment in the two groups.

Table 4 Incidence of post-gonococcal urethritis (PGU)

Group	No. treated	No. assessed for PGU	Time of detection of PGU			Total	% followed up
			Second week	Third week	Fourth week		
A	109	84	18	4	3	25	29.8
B	101	82	20	6	3	29	35.4

Table 5 Penicillin sensitivities in the two groups in µg/ml

Group	No. tested	0.06 or less	0.125	0.25	0.5	>0.5	% sensitive to 0.125 or more
A	95	71	5	13	5	1	25.3
B	85	69	11	3	2	0	18.8

Table 6 Ampicillin sensitivities in the two groups in µg/ml

Group	No. tested	0.06 or less	0.125	0.25	0.5	>0.5	% sensitive to 0.125 or more
A	95	68	16	9	1	1	28.4
B	85	61	15	9	0	0	28.2

Table 7 *Penicillin and ampicillin sensitivities of gonococci grown from patients with subsequent recurrences*

Group	Time of recurrence	Sensitivities (μg/ml)				No data	Total
		0.06 or less		0.125 or more			
		Ampicillin	Penicillin	Ampicillin	Penicillin		
A	Week						
	First	2	2	0	0	0	
	Second	0	0	0	0	0	8
	>2 weeks	4	5	2	1	0	
B	Week						
	First	2	2	1	1	0	
	Second	0	0	0	0	0	13
	>2 weeks	6	7	2	1	2	

The incidence of side effects was small. There were no allergic reactions, mainly because all patients had been questioned carefully about any history of allergy to penicillin.

The number of strains of *N. gonorrhoeae* sensitive to 0.125 $\mu\text{g/ml}$ or more of penicillin at The London Hospital in 1968-69 was 19.9% (164 of 825 strains). This figure had increased to 32.9% (72 of 228 strains) in 1972 (Rodin and Seth, 1972) and was 30% (83 of 227 strains) in 1975 (Taylor and Seth, 1975). The current figure of 22.2% (40 of 180 strains) suggests a shift towards more sensitive strains in the last three years. This may be related to the policy of treatment with high doses used in recent years (Olsen and Lomholt, 1969).

We thank Mrs Astrid Forse of the Venereal Disease Reference Laboratory, and Mr S. Goldsmith and the nursing staff for their help in this trial.

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Changes in the incidence of acute gonococcal and nongonococcal salpingitis

A five-year study from an urban area of central Sweden

LARS FORSLIN, VIKING FALK, AND DAN DANIELSSON

From the Department of Obstetrics and Gynaecology, and the Department of Clinical Bacteriology and Immunology, Central County Hospital, S-701 85 Örebro, Sweden

SUMMARY The incidence of acute gonococcal and nongonococcal salpingitis for a five-year-period (1970-74) was studied retrospectively in an urban area of central Sweden. The investigation was undertaken to see if the reported decrease of gonorrhoea in Sweden had been followed by a change in the incidence of gonococcal salpingitis—the most common complication of gonorrhoea. The study showed that the relative incidence of acute gonococcal salpingitis had decreased even more than urogenital gonorrhoea and these findings thus indicate a real decrease of gonorrhoea. At the same time there were more patients with nongonococcal salpingitis. During the period of the study the gonococcal complement-fixation test (GCFT) gave positive results in 40% to 80% of the patients with gonococcal salpingitis. The yield with this test was only 4% in patients with nongonococcal salpingitis during 1970 but it increased successively and was 23% in 1974. This increase was statistically highly significant ($P < 0.001$).

Introduction

In the last 15 years there has been a steady increase in the incidence of gonorrhoea and an increase of as much as 15% each year was reported from USA and Sweden during the late 1960s. This trend continued in USA during the early 1970s (Wiesner and Holmes, 1975). A similar situation was described from Denmark and the United Kingdom but a levelling off has now been reported (Catterall, 1975; Juhlin, 1975; Morton, 1975). In Sweden, however, a pronounced decrease took place in 1971-72 and this continued during the next four years with figures in 1976 corresponding with those reported in 1965 (Juhlin, 1975; Kallings and Moberg, 1977).

The reasons for this decrease are not clear. Under-reporting by doctors is one explanation, but this has not been substantiated. If this were true the relative incidence of complications would increase. We therefore tested this hypothesis by studying the incidence of acute salpingitis with concomitant urogenital gonorrhoea, so-called gonococcal salpingitis, which several investigations have

shown to be the most common complication of urogenital gonorrhoea. The incidence in the United Kingdom has been reported as being between 10 and 15% (Rees and Annels, 1969) and a similar incidence has been reported in various areas of Sweden during the 1950s and 1960s (Gisslén *et al.*, 1961; Falk, 1965; Mårdh and Weström, 1970; Danielsson *et al.*, 1975). Acute salpingitis without concomitant urogenital gonorrhoea is called nongonococcal salpingitis.

We made a retrospective investigation of the incidence of acute gonococcal and nongonococcal salpingitis during the period 1970-74 in the district of Örebro city, an urban area of central Sweden. We found that the relative incidence of acute gonococcal salpingitis decreased even more than urogenital gonorrhoea. At the same time more patients had nongonococcal salpingitis.

Material and methods

PATIENTS AND DIAGNOSTIC CRITERIA

The study comprised 666 inpatients from the Department of Gynaecology, Central County Hospital, Örebro, Sweden, in whom acute salpingitis had been diagnosed. During the period 1970-74 any patient attending the hospital with a suspected

Address for reprints: Lars Forslin, Department of Obstetrics and Gynaecology, Central County Hospital, S701 85 Örebro, Sweden

Received for publication 14 December 1977

diagnosis of acute salpingitis was routinely admitted to hospital.

The diagnostic criteria were:

A history of low abdominal pain with palpable adnexal mass and at least two of the following symptoms: vaginal discharge, menstrual irregularity, dysuria, temperature $>38^{\circ}\text{C}$, and an erythrocyte sedimentation rate >15 mm/1st hour. The physical examination was carried out and the presence of a palpable adnexal mass was confirmed by one or two senior physicians on the morning of the day after the patient had been admitted to hospital. In 447 (67.1%) of the patients a diagnosis of acute salpingitis was confirmed by visual inspection by laparoscopy using the criteria laid down by Jacobson and Weström (1969). Laparoscopy was used on any patient in whom the diagnosis was uncertain on clinical grounds. Anyone who did not fulfil the criteria or who gave negative results on laparoscopy was excluded.

BACTERIOLOGY AND SEROLOGY

On the day the patient was admitted to hospital, or on the morning of the next day when the patient was again examined, specimens for culture of gonococci were taken from the urethra, cervical os, and the rectal mucosa with charcoal-treated cotton swabs and transported to the laboratory in modified Stuart's transport medium (Gästrin and Kallings, 1968). On arrival at the laboratory (within 4–16 hours) the specimens were cultured on selective and nonselective gonococcal culture media and then *Neisseria gonorrhoeae* were isolated and identified as earlier described (Danielsson and Johannisson, 1973).

A serum specimen was taken from each patient within one day of admission to the hospital. A second serum specimen was obtained between two and three weeks later from 80% of the patients. The sera were tested for gonococcal antibodies using the micromodification of the Laboratory Branch Complement Fixation (LBCF) test as described elsewhere (Danielsson *et al.*, 1972). This technique has been used in our laboratory for the last nine years, and a titre of $>1:5$ is regarded as positive.

Statistical analysis

The hypothesis was tested by the χ^2 test. The following terms were used: $P < 0.001$ difference highly significant; $0.001 < P < 0.01$, difference significant; $0.01 < P < 0.05$, difference almost significant.

Results

AGE DISTRIBUTION OF PATIENTS WITH ACUTE SALPINGITIS AND THE INCIDENCE OF UROGENITAL GONORRHOEA

The Figure shows the age distribution of the patients with acute salpingitis with or without concomitant urogenital gonorrhoea. It will be seen that most cases were in patients aged between 15 and 24 years. There were, however, more patients aged between 30 and 34 years during 1973–74 compared with 1970–71, but this increase was not statistically significant.

The total number of patients with acute salpingitis was relatively constant in our particular area of central Sweden during 1970–73 with an increase in 1974 (Figure and Table 1). However, the number of

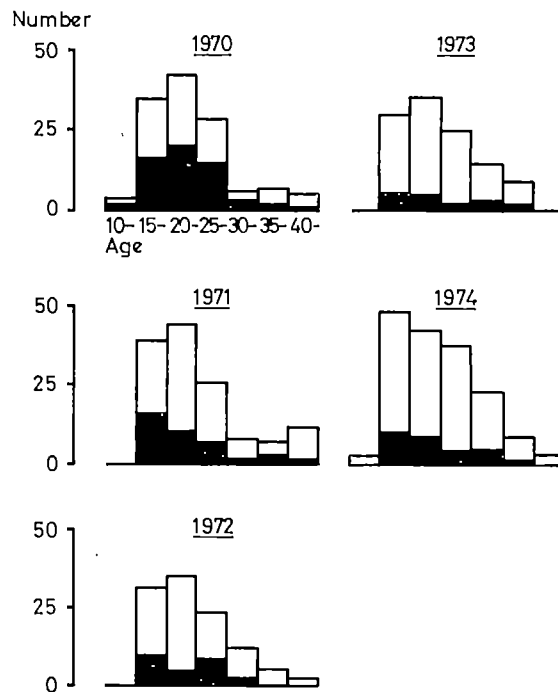


Table 1 The incidence of urogenital gonorrhoea in patients admitted to hospital with acute salpingitis

Year	Acute salpingitis			Probability (1970)
	Total No.	With gonorrhoea No.	%	
1970	126	54	42.9	—
1971	139	38	27.3	$P < 0.01$
1972	111	24	21.6	$P < 0.001$
1973	113	18	15.9	$P < 0.001$
1974	177	30	16.9	$P < 0.001$

patients with acute salpingitis with concomitant urogenital gonorrhoea decreased to a significant extent compared with those without gonorrhoea. Table 1 shows that the incidence of urogenital gonorrhoea was 42.9% in these patients in 1970 which is in agreement with earlier reports (Falk, 1965; Danielsson *et al.*, 1975) but it was only 15.9% and 16.9% respectively in 1973 and 1974 ($P < 0.001$).

EPIDEMIOLOGICAL SITUATION AND CALCULATION OF THE RELATIVE INCIDENCE OF ACUTE SALPINGITIS IN WOMEN WITH GONORRHOEA

Doctors in Sweden are bound by law to report to public health authorities patients who are diagnosed and being treated for gonorrhoea. We therefore obtained from the files the number of women with gonorrhoea who came from the same central area as those in hospital. This gave us an opportunity to calculate the relative incidence of acute salpingitis in patients with urogenital gonorrhoea (Table 2). The number of women with urogenital gonorrhoea markedly decreased during the period of our study. At the same time the number of patients with acute salpingitis and concomitant urogenital gonorrhoea decreased even more. This decrease was particularly obvious in 1973 and almost significant compared with 1970 ($0.05 > P > 0.01$). However, the relative incidence increased in 1974 to approximately the same level as in 1970.

Table 2 *The yearly number of women in the area studied in whom gonorrhoea was diagnosed and reported and the relative incidence of acute salpingitis in these patients*

Year	Reported gonorrhoea in women			Probability related to 1970
	Total No.	With salpingitis No.	%	
1970	507	51*	10.6	—
1971	409	35	8.7	$P > 0.05$
1972	307	19	6.2	$P > 0.005$
1973	292	17	5.8	$0.05 > P > 0.01$
1974	226	24	10.3	$P > 0.05$

*Figures not comparable with those given in Table 1 because of correction for the particular area we studied.

YIELD WITH THE GCFT

The yield with the gonococcal complement fixation test (GCFT) in patients with acute gonococcal and nongonococcal salpingitis respectively is shown in Table 3. It will be seen that 40–50% of the patients with gonococcal salpingitis had a positive GCFT in 1970–73 and 83% of them had a positive GCFT in 1974. Table 3 also shows that the yield with the

GCFT in patients with nongonococcal salpingitis increased from 4% in 1970 to 23% in 1974. This increase is highly significant ($P < 0.001$).

Table 3 *The yield with the gonococcal complement fixation test (GCFT) in patients with acute gonococcal or nongonococcal salpingitis*

Year	Patients with acute salpingitis					
	With gonorrhoea			Without gonorrhoea		
	Positive GCFT No.	%	Neg. GCFT No.	Positive GCFT No.	%	Neg. GCFT No.
1970	25	47	29	3	4	69
1971	15	40	23	9	9	92
1972	13	54	11	11	12	76
1973	7	39	11	17	18	78
1974	25	83	5	33	23	114

Discussion

This investigation showed that the incidence of urogenital gonorrhoea decreased in our area as it did in the whole of Sweden during the first part of the 1970s (Kallings and Moberg, 1977). At the same time the incidence of acute gonococcal salpingitis also decreased and to a greater extent than gonorrhoea in females. If the decrease of gonorrhoea had been due to under-reporting, which was a possible explanation, the incidence of gonococcal salpingitis would have increased. Our findings therefore show that there was an actual decrease in gonorrhoea in Sweden during the first half of the 1970s. This is further supported by the fact that the incidence of urogenital gonorrhoea in patients admitted to hospital with acute salpingitis dropped from nearly 43% in 1970, to approximately 16% in 1973, and to 17% in 1974.

Despite the decrease of gonococcal salpingitis the number of patients with acute salpingitis remained unchanged and even increased during the last year of our study. This increase of nongonococcal salpingitis was in all age groups, and the reasons for it are not clear. It might have been caused by recurrences but our data do not confirm this. Nor can it be explained by other criteria being used for admitting patients with a suspected diagnosis of acute salpingitis as the same criteria were used for the whole investigation. An analysis of the type of contraceptives used showed that more patients used intrauterine devices (IUDs) during 1973–74. Thus, during 1970, 1971, and 1972 respectively only 2.3%, 3%, and 7% of the patients used this type of contraceptive, but in 1973 and 1974 respectively as many as 26% and 29% of the patients had IUDs. Weström *et al.* (1976) noted a higher incidence of acute salpingitis among patients with IUDs. These

circumstances might have contributed to the relative increase of acute nongonococcal and gonococcal salpingitis noted during 1974, but it cannot explain the relative increase of nongonococcal salpingitis during 1970, 1971, and 1972.

It is also tempting to speculate whether the increase could be related to a higher incidence of non-specific urethritis (NSU) in men. Unfortunately, during the period of this study NSU was not reported in Sweden or in other Scandinavian countries. We therefore have no statistics on the incidence of NSU among men although the general impression among Swedish venereologists is that this disease has increased during the 1970s and that it now occurs nearly twice as often as gonococcal urethritis.

We have no data concerning pathogens other than gonococci. It was of interest to note, however, that the positive yield with the GCFT increased among the patients with nongonococcal salpingitis and it was highly significant in 1974 compared with 1970. This increase is not caused by laboratory methods as the same technique for preparing antigens and performing the test has been used for the last 10 years in our laboratory. These findings are interesting from a pathological point of view since it has been proposed by Blinick (1959) that a gonococcal salpingitis could precede infection with other micro-organisms. The increase of positive GCFT results among patients with nongonococcal salpingitis may therefore be due to an earlier gonococcal infection that has preceded invasion with other micro-organisms. In this connection it is of interest to note that the positive yield with the GCFT among patients with gonococcal salpingitis increased from approximately 50% in 1972 and 1973 to 83% in 1974 and at the same time there were more patients with acute salpingitis and gonorrhoea. One could therefore speculate on the possibility of certain gonococcal strains having a greater tendency to induce salpingitis than others. Investigations are now under way to study these suggestions.

This work was in part supported by the World Health Organisation.

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Chlamydia trachomatis in non-specific urethritis

PERTTI TERHO

From the Department of Virology, and the Department of Dermatology and Venereology,
University of Turku, Finland

SUMMARY *Chlamydia trachomatis* was isolated from 58.5% of 159 patients with non-specific urethritis (NSU) using irradiated McCoy cell cultures. Patients with persistent *Chlamydia*-positive NSU remained *Chlamydia*-positive each time they were examined before treatment and patients with *Chlamydia*-negative NSU remained *Chlamydia*-negative during the course of the illness. Neither the duration of symptoms of urethritis nor a history of previous urethritis affected the chlamydial isolation rate significantly. Of 40 patients with severe discharge 30 (75%) harboured *C. trachomatis*. One-third of the *Chlamydia*-positive patients had a severe urethral discharge, while this was present in only 15% of *Chlamydia*-negative patients. Complications—such as conjunctivitis, arthritis, and epididymitis—were more severe in men with *Chlamydia*-positive NSU than in those with *Chlamydia*-negative NSU. Of 64 men matched for sexual promiscuity but without urethritis, none harboured *C. trachomatis* in his urethra. This differs significantly ($P < 0.001$) when compared with patients with NSU. *C. trachomatis* was isolated from the urogenital tract in 24 (42%) out of 57 female sexual contacts of patients with NSU. The presence of *C. trachomatis* in the women correlated significantly ($P < 0.001$) with the isolation of the agent from their male contacts. These findings give further evidence for the aetiological role of *C. trachomatis* in non-specific urethritis and its sexual transmission.

Introduction

Sexually transmitted urethritis in men is either gonococcal or nongonococcal. In Britain (where both types are reported) nongonococcal urethritis (NGU) is now more common than gonococcal urethritis. NGU accounts for more than two-thirds of all urethritis in men and is increasing more rapidly than gonococcal urethritis (Department of Health and Social Security, 1977).

In fewer than 10% of cases of NGU it is possible by the use of routine laboratory techniques to find a specific cause—such as *Trichomonas vaginalis*, *Candida albicans*, or herpesvirus hominis. The remainder of NGU is called non-specific urethritis (NSU) (Dunlop, 1975). NSU usually causes symptoms and signs that are less severe than those of gonococcal urethritis, and these may even be absent (Rodin, 1971; Oriel, 1976). NSU is diagnosed by demonstrating an inflammatory reaction in the urethra and by excluding specific causes—especially gonorrhoea (Oriel, 1976).

About 12 years ago *Chlamydia trachomatis* was first isolated from the urethra of patients who presented with NSU (Dunlop, 1965). *C. trachomatis* has since been shown to be one of the main causes of NSU (Dunlop, 1975; Oriel, 1976). It was isolated using sophisticated cell culture methods (Dunlop, 1975) from over 40% of patients with NSU and from many of their female sexual partners (Richmond and Sparling, 1976; Schachter *et al.*, 1976). In control groups of men matched for sexual activity but without urethritis the chlamydial isolation rate was 0 to 7% (Oriel, 1976).

In the present study, the isolation of *C. trachomatis* in a series of 159 men with NSU is reported, and the clinical findings of NSU are analysed in relation to the isolation of *C. trachomatis*. The isolation results in a control group of 64 men without urethritis and in 57 female sexual partners of patients with NSU are also presented.

Material and methods

STUDY POPULATION

The study population consisted of unselected Caucasian heterosexual men presenting either at the Turku Venereal Disease Clinic or the Depart-

Address for reprints: P. Terho MD, Department of Virology, University of Turku, Kilnamyllynkatu 10, 20520 Turku 52, Finland

Received for publication 1 November 1977

ment of Dermatology and Venereology, University of Turku, between December 1973 and May 1976. Patients were included in the study provided they had not received treatment with antimicrobial agents within the previous six weeks. Each man had negative results to a urethral culture for *Neisseria gonorrhoeae*. The patients were divided into two groups.

NSU group

This group initially comprised 164 men. All had microscopical evidence of urethritis with more than 20 polymorphonuclear leucocytes in two fields or more on a Gram-stained urethral smear examined under a magnification of $\times 800$. Specimens for chlamydial isolation from five (3%) of these men were contaminated, so these patients were excluded from the study. The mean age of the remaining 159 patients was 27.5 years.

Control group

This group initially comprised 68 men who were attending the same clinic but who did not have urethritis. Specimens from four (6%) of them were invalid either because of contamination with bacteria or because of toxicity for cells. Consequently the control group comprised 64 men, whose mean age was 29.9 years. Their marital state was comparable with that of the patients with NSU.

Female sexual partners of NSU patients

Sixty female sexual partners of patients with NSU were examined. Three (5%) of them (all consorts of patients with *Chlamydia*-positive NSU) had contaminated specimens, so 57 women were included in the study.

CLINICAL EVALUATION

A standardised history was taken, special attention being paid to previous venereal diseases, recent sexual partners, possible incubation time, and duration of symptoms. General medical and genital examinations were carried out in all cases. Eyes, joints, mucous membranes, and genitalia were carefully examined to see if there were complications. Most of the patients were studied about four hours after passing urine, but in some the overnight urethral secretion was examined to reveal low-grade urethritis. Some patients had at least two examinations before medical treatment was begun. Patients were asked to return for examinations within one week of completing treatment, and then at one-monthly and two-monthly intervals. At each visit the clinical state was recorded, and relevant specimens were taken.

SPECIMENS

Urethral secretions produced spontaneously or by massaging the urethra towards the meatus were smeared with a sterile platinum loop on to a slide and stained by Gram's method. For gonococcal culture, secretions from the urethra were smeared directly with the loop on Müller-Hinton agar and/or on Columbia agar plates, which were immediately incubated in a candle-jar at 37°C. Gonococci were identified by conventional bacteriological techniques with sugar fermentation tests. All gonococcal cultures were processed at the Department of Medical Microbiology, University of Turku.

Specimens for isolating *C. trachomatis* were obtained with endourethral swabs inserted 4 cm from the meatus. The swabs were placed in 2-sucrose phosphate (2 SP) and subsequently inoculated on to irradiated McCoy cells. The technique used for isolating *C. trachomatis* is described in detail elsewhere (Terho, 1978). Briefly, irradiated McCoy cells grown on a coverglass in flat-bottomed tubes were infected by centrifugation (15 000 *g* for 1 hour at 35°C). After incubation for 72 hours in a CO₂ atmosphere at 35°C, cells were stained with iodine, and chlamydial inclusions were identified by light microscopy.

Urethral secretions from some patients were cultured for herpesvirus hominis by inoculation on to Vero cells. No attempt to cultivate mycoplasmas or ureaplasmas was made. Specimens for chlamydial isolation were taken from the urethra and from the cervical canal in women.

Statistics

Statistical analysis was made by the *t* test or χ^2 test (with Yates's correction if appropriate). Probabilities were classified as almost significant ($P < 0.05$), significant ($P < 0.01$), or highly significant ($P < 0.001$).

Results

ISOLATION OF *C. TRACHOMATIS*

C. trachomatis was isolated from 93 (58.5%) of 159 men with NSU. In no man without urethritis was *C. trachomatis* isolated. This difference is highly significant (χ^2 61.8; $P < 0.001$).

REPEATED ISOLATION OF *C. TRACHOMATIS*

Before treatment at least two specimens were taken for culture for *C. trachomatis* from 23 unselected men; of these 13 gave *Chlamydia*-positive results and 10 *Chlamydia*-negative. All except four men showed persistent NSU on re-examination; two of them were *Chlamydia*-positive at the initial visit but free of urethritis and *Chlamydia*-negative six

The numbers of inclusion-forming units (IFU) in specimens taken repeatedly before treatment were constant; in four instances there was some increase. In five there was a slight decrease, and in two the numbers were the same.

In the control group NSU was almost significantly less common (10 out of 64 or 15%) than in the NSU group, in which 49 out of 159 (31%) had had NSU earlier (χ^2 5.41, $P < 0.05$). The history of previous gonococcal urethritis was equally common in the NSU and control groups.

Patients with acute NSU usually presented after a recent change of sexual partner; the mean duration of symptoms at attendance was 11.1 days in the

Group	Chlamydia Isolation	No.	%	Previous urethritis											
				No previous urethritis		GU		NSU		GU+NSU		Unknown		Total	
				No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
NSU	Positive	93	(58.5)	38	(41)	21	(23)	20	(22)	12	(13)	2	(2)	55	(59)
	Negative	66	(41.5)	29	(44)	18	(27)	11	(17)	6	(9)	2	(3)	37	(56)
	Total	159	(100)	67	(42)	39	(25)	31	(19)	18	(11)	4	(3)	92	(58)
Control	Negative	64	(100)	39	(61)	15	(24)	6	(9)	4	(6)	—	—	25	(39)

100 men in whom it was possible to determine this. The duration of symptoms was slightly longer in the *Chlamydia*-positive group (mean 12.8 days) than in the *Chlamydia*-negative group (mean 9.8 days), but this difference does not reach statistical significance (t 1.39). Short duration of symptoms (less than seven days) was recorded in 42 (26.4%) of the 159 cases with NSU. Twenty-three (54.8%) of these 42 men had positive *Chlamydia* culture results, and 19 (45.2%) had negative results, rates similar to those of the whole (NSU) group.

Chronic NSU

Duration of urethral symptoms for longer than 30 days was observed in 26 (16.4%) patients with NSU who did not have a recent change of sexual partner. Most of them (18 or 69%) harboured *Chlamydia*.

TIME FROM PREVIOUS SEXUAL INTERCOURSE

It was possible to determine the time from the previous casual sexual contact or the last sexual intercourse with a regular partner to the onset of symptoms for 86 patients with NSU. The mean time was 12.4 days. There was no difference in this between patients with positive and negative *Chlamydia* culture results.

In the control group the time from previous sexual intercourse averaged 6.3 days. This interval is significantly shorter than in the NSU group (t 3.66, $P < 0.001$).

TIME FROM LAST MICTURITION

Most patients were examined more than two hours after micturating. In the NSU groups 39 (25%) patients were examined less than two hours after passing urine, but it was possible to diagnose NSU in most of them; only five patients (three *Chlamydia*-positive and two *Chlamydia*-negative) had their overnight urethral secretion investigated so that low-grade NSU could be diagnosed. In the control group 18 (28%) men were examined within two hours of micturating. Because of practical difficulties it was not possible to investigate their overnight urethral secretion, so some cases of urethritis may have been missed (Rodin, 1971).

AMOUNT OF DISCHARGE

The amount of urethral discharge was determined as 'severe' if frank, spontaneous discharge was present. The discharge was classified as 'slight' if the urethra had to be massaged or if only a small droplet of discharge appeared spontaneously at the meatus. In patients with no discharge it was only possible to obtain the contents of the urethra by using a platinum loop. The amount of discharge is shown in Table 2.

Table 2 Isolation of *C. trachomatis* and the amount of urethral discharge

Amount of discharge	Isolation of <i>C. trachomatis</i>		
	Positive	Negative	Total
None	10	14	24
Slight	53	42	95
Severe	30	10	40
Total	93	66	159

Of the *Chlamydia*-positive patients 30 (32%) had severe discharge, while only 10 (15%) of the 66 *Chlamydia*-negative patients had. This difference is almost significant (χ^2 6.00, $P < 0.05$).

Thus a total of 40 patients with NSU had severe discharge, and of them 30 (75%) were *Chlamydia*-positive. When comparing this finding with patients with slight discharge, of whom 56% (53 out of 95) were *Chlamydia*-positive, it can be stated as almost significant (χ^2 4.39, $P < 0.05$).

COMPLICATIONS OF NSU

Chlamydia-positive NSU

Two patients had isolation-positive chlamydial conjunctivitis in addition to chlamydial urethritis. Two additional patients had arthritis of the knee concomitant with chlamydial urethritis; in one of them we attempted to isolate *C. trachomatis* from the joint fluid but the result was negative. One patient developed severe epididymitis after chlamydial NSU of long duration.

Chlamydia-negative NSU

Two patients developed a slight *Chlamydia*-negative conjunctivitis at the time of NSU. One patient had a mild epididymitis. One patient presented with Reiter's disease, which he had had twice before.

ISOLATION OF *C. TRACHOMATIS* FROM FEMALE PARTNERS

Twenty-four (42%) out of 57 female sexual partners of patients with NSU were *Chlamydia*-positive (Table 3). Of these 35 were partners of men harbouring *C. trachomatis*; the agent could be isolated from the urogenital tract of 22 (63%) (Table 3). Chlamydial isolation was possible only in two (9%) of the 22 partners of *Chlamydia*-negative patients with NSU. This difference is highly significant (χ^2 13.9, $P < 0.001$). The cervix gave positive results

Table 3 Isolation of *C. trachomatis* from female sexual partners

		Chlamydia in men	
		Positive	Negative
Chlamydia in women			
Positive	24	22	2
Negative	33	13	20

in 20 (83%) out of a total of 24 *Chlamydia*-positive women. Urethral isolation was positive in 14 cases (58%) and the urethra was the only site of recovery in four (17%) *Chlamydia*-positive cases.

Discussion

C. trachomatis can be isolated significantly more often from men with NSU than from control men without urethritis (Richmond and Sparling, 1976; Schachter *et al.*, 1976). In this study the *C. trachomatis* isolation rate was 58.5% for patients with NSU. This figure is very close to the 60% suggested by Oriel *et al.* (1976) as the optimal rate for current techniques.

Repeated examinations before treatment or after treatment with placebo show that NSU may disappear spontaneously. Handsfield *et al.* (1976) recorded resolution or improvement of NSU after placebo in five out of 23 patients. In the present study NSU disappeared in four of 23 patients when repeated examinations were made.

If urethritis remained, repeated isolation of *C. trachomatis* was also possible. Thus all 11 *Chlamydia*-positive patients remained so, and all eight *Chlamydia*-negative patients remained negative on repeated examinations, which may be a proof of the sensitivity of the present isolation method. This finding is in accordance with the repeated isolations in the study of Holmes *et al.* (1975). It shows also that *C. trachomatis* does not usually disappear spontaneously and gives support for the treatment of this urethral pathogen. The present study is in agreement with previous ones (Oriel, 1976) in the absence of chlamydial isolations from men without urethritis even if their sexual promiscuity was equal to that of patients with NSU.

Patients with NSU in this study had had urethritis before (especially NSU) significantly more often than had the controls. In the NSU group the type of previous urethritis did not affect the isolation rate of *C. trachomatis*. This finding agrees with that of Oriel *et al.* (1976). Recently, however, Alani *et al.* (1977) reported significantly lower isolation rates of *C. trachomatis* from men with a third episode of NSU. They suggested some immunological phenomenon as the cause of reduced isolation rate.

Men with NSU may have no symptoms (Rodin, 1971). In the present study 10% of all patients with NSU were unaware of their urethritis. The incubation time of NSU is longer than that of gonococcal urethritis. The mean time (recorded from the previous sexual intercourse) of 12.4 days in this study correlates well with that reported by Oriel *et al.* (1976); they found that the incubation time in

Chlamydia-positive patients was 16 days and in negative ones 12 days.

Duration of symptoms before attendance had a mean of 11.1 days in those patients from whom this could be determined. This time interval did not have any influence on chlamydial isolation rates, in contrast to the report of Oriel *et al.* (1976) who found significantly higher isolation rates in patients in whom symptoms lasted more than seven days.

The amount of urethral discharge seems to correlate with chlamydial isolation rates, although the isolation was possible from men without apparent discharge. One-third of all patients with *Chlamydia* had a severe discharge. Among a selected group of patients with NSU with severe discharge 75% were *Chlamydia*-positive. This finding is similar to those of Alani *et al.* (1977), who found that chlamydial isolation was positive in 67.7% of men with severe discharge.

The clinical picture is comparable in *Chlamydia*-positive and *Chlamydia*-negative NSU (Oriel *et al.*, 1976), but in the present study *Chlamydia*-positive NSU was associated with more severe complications. *Chlamydia*-positive conjunctivitis was recorded in two patients with *Chlamydia*-positive NSU and also in one female partner of a man with *Chlamydia*-positive NSU.

Joint complications, including painful arthritis of the knee in two patients, were also more severe in the *Chlamydia*-positive NSU group. One severe case of epididymitis occurred in the *Chlamydia*-positive group and one mild case of epididymitis in the *Chlamydia*-negative group. Heap (1975) reported two cases of acute chlamydial epididymitis, one of which led to surgery. Recently, Harnisch *et al.* (1977) showed that *C. trachomatis* may be the cause of acute epididymitis in young men as often as *N. gonorrhoeae*.

The isolation rate of 42% of *C. trachomatis* from female partners of patients with NSU is slightly higher than that of other studies. Hilton *et al.* (1974) found 34%, and Nayyar *et al.* (1976) 30% of partners of patients with NSU harbour *Chlamydia*.

The sexual transmissibility of *C. trachomatis* was shown by the demonstration of the agent significantly more often in the urogenital tract of partners of patients with *Chlamydia*-positive NSU. This agrees well with results of Alani *et al.* (1977).

The author thanks Ms Ulla Virtanen and Ms Anni Heikkilä for their help during clinical work and Ms Tuula Lindholm for her excellent technical assistance. Dr E. M. C. Dunlop is thanked for many valuable suggestions during the preparation of this manuscript.

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Genital chlamydial infections in patients attending a gynaecological outpatient clinic

J. PAAVONEN,* P. SAIKKU,† E. VESTERINEN,* B. MEYER,*
E. VARTIAINEN,* AND E. SAKSELA*

From the *I-II Departments of Obstetrics and Gynaecology, University Central Hospital, Helsinki, Finland, and †Department of Virology, University of Helsinki, Helsinki, Finland

SUMMARY Investigation for genital chlamydial infections was carried out on 160 patients of fertile age attending a gynaecological outpatient clinic. Thirteen (9%) of the 144 patients with valid specimens were found to be *Chlamydia*-positive. Seven (54%) of them had antichlamydial antibodies by the complement-fixation test, and 11 (85%) antichlamydial antibodies by a single-antigen immunofluorescence test compared with 21 (16%) and 70 (53%), respectively, of the 131 *Chlamydia*-negative patients. Patients with overt genital chlamydial infection often complained of ocular and urethral symptoms. Usually there was clinical evidence of cervical infection as well as of inflammation on cervicovaginal smears. Colposcopic and histological examinations did not show any specific alterations.

Introduction

Chlamydia trachomatis is an obligate, intracellular parasite causing trachoma, paratrachoma, lymphogranuloma venereum, and genitourinary infections in humans (Grayston and Wang, 1975; Schachter *et al.*, 1976). *C. trachomatis* is recognised as common in the genital tract of women attending clinics for venereal diseases as well as of the sexual partners of men with nongonococcal urethritis (NGU) (Richmond and Sparling, 1976; Schachter *et al.*, 1976; Alani *et al.*, 1977; Rees *et al.*, 1977).

Cervicitis and pelvic inflammatory disease in women may be aetiologically related to *Chlamydia* (Schachter *et al.*, 1967; Eschenbach *et al.*, 1975; Schachter *et al.*, 1975a; Mårdh *et al.*, 1977; Rees *et al.*, 1977). Because it may possibly be associated with cervical malignancy (Alexander, 1973; Schachter *et al.*, 1975b), this agent has also attracted attention as an important infective agent. Although chlamydial infections are not yet proved causes of cervical disease (Richmond and Sparling, 1976), it has been suggested that *Chlamydia* may make specific morphological alterations to cervical epithelial cells (Naib, 1970; Dunlop *et al.*, 1976). Women with genital chlamydial infection often show a serologically detectable immunological

response towards antigens of the organism (Richmond and Caul, 1975, 1977).

The aims of the present work were to study prospectively the incidence and nature of genital chlamydial infection in a sample population from a general gynaecological outpatient clinic and to evaluate the diagnostic value of the methods applied in the gynaecological routine.

Material and methods

PATIENTS

The sample population consisted of 160 patients of fertile age attending the gynaecological and obstetric outpatient clinics at Helsinki University Central Hospital in September and October 1976.

The patients did not have acute symptoms but were either being examined for gynaecological disease or were under observation for hormonal, fertility, or similar problems. Postmenopausal patients and those being treated with antibiotics or sulphonamides were excluded. The mean age of the patients was 29.5 years (range 17-49). Thirty-nine patients were fitted with intrauterine devices, 22 were taking oral contraceptives, and 81 used other methods or none at all, and 18 were pregnant.

CHLAMYDIAL ISOLATION AND SEROLOGY

Specimens for chlamydial isolation were collected with a sterile cottonwool swab from the endocervix

Address for reprints: J. Paavonen, MD, I Department of Obstetrics and Gynaecology, University Central Hospital, Haartmaninkatu 2, Helsinki, Finland 00290

Received for publication 3 January 1978

into two SP transport media (Dunlop *et al.*, 1972). Isolation attempts were done conventionally in irradiated McCoy cells (Darougar *et al.*, 1971). Gentamicin was used as antibiotic.

Serum antibodies were determined by complement-fixation (CF) and indirect immunofluorescence (IF) tests. CF group antigen was prepared by the acetone-ether method from *C. trachomatis* (kindly supplied by Dr C. Mordhorst, Statens Seruminstitut, Copenhagen, Denmark) and the test was done conventionally in a microsystem. For the IF test the following method modified from the original one (Dr P. Terho, Department of Virology, University of Turku) was used. The antigen slides were prepared by growing continuous untreated epithelioid dog kidney cells (kindly supplied by Dr N. Oker-Blom, Department of Virology, University of Helsinki) on slides in Petri dishes. The cells were infected with *C. trachomatis* serotype L2 to produce inclusions acting as the antigen in the test (Saikku and Paavonen, 1978).

CYTOLOGICAL AND HISTOLOGICAL TECHNIQUES

The cytological smears obtained from the posterior vaginal fornix, the ectocervix, and the endocervix were stained according to Papanicolaou's original method. Histological samples from *Chlamydia*-positive patients were obtained at colposcopy, fixed in formalin, and stained with van Gieson-haematoxylin or haematoxylin-eosin using routine methods.

Colposcopy was performed with a Zeiss-Oberkochen colposcope using magnification $\times 40$. Colposcopic classification of three grades was used as described in detail elsewhere (Timonen and Meyer, 1967).

VIROLOGICAL TEST AND ISOLATION OF OTHER MICRO-ORGANISMS

Herpes genitalis was diagnosed from virus isolation specimens obtained from the cervix (Leinikki and Salo, 1973), or from characteristic alterations

observed at the routine examination of the cytological smears, or from both (Naib *et al.*, 1966; Vesterinen *et al.*, 1977).

Genital condyloma accuminatum was diagnosed both clinically and cytologically (Purola and Savia, 1977). Cultivation of *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, and yeast, as well as urine analysis for bacterial infection, was made by conventional methods (Lennette *et al.*, 1974).

Results

Thirteen (9%) of the 144 patients with valid isolation specimens harboured *Chlamydia*. In 16 patients the samples were contaminated by an overgrowth of mixed microbial flora.

Chlamydial group-specific antibodies determined by the CF test at a dilution of 1:8 or more were detected in seven (54%) of the 13 *Chlamydia*-positive patients and in 21 (16%) of the 131 *Chlamydia*-negative patients. *Chlamydia* group-specific antibodies at a level of 1:8 or more determined by a single-antigen IF test were detected in 11 (85%) of the *Chlamydia*-positive patients and in 70 (53%) of the *Chlamydia*-negative patients. IF test titres $\geq 1:64$ occurred in eight (62%) of *Chlamydia*-positive patients and in 25 (19%) of *Chlamydia*-negative patients (Table 1). The Figure compares antibodies detected by the CF and IF tests in sera from 13 *Chlamydia*-positive patients. Within two months one isolation-positive patient seroconverted ($\geq$ fourfold rise in titre) by CF test and four patients by IF test.

Eight (62%) of the 13 *Chlamydia*-positive patients showed clinical cervicitis with or without cervical erosion (inflammation and hypertrophy of the cervix with mucopurulent discharge) compared with 43 (33%) of the 131 *Chlamydia*-negative patients. Thus, 16% of the 51 patients with cervical infection, 9% of the 22 patients with vaginitis only (inflamed vaginal mucosa and leucorrhoea without cervical infection), and 4% of the 71 patients with no cervicovaginal abnormalities harboured *Chlamydia*. How-

Table 1 *Chlamydial antibody titres found in CF and IF tests of sera from patients attending a gynaecological outpatient clinic*

No. of sera	<8	8	16	32	64	128	256	512	1024	Total ≥ 8 No.	%	Total ≥ 64 No.	%
<i>Chlamydia</i> -positive*													
CF 13	6	5	0	2	0	0	0	0	0	7	54	0	
IF 13	2	0	1	2	2	2	3	0	1	11	85	8	62
<i>Chlamydia</i> -negative													
CF 131	110	10	8	3	0	0	0	0	0	21	16	0	
IF 131	61	23	10	12	11	9	5	0	0	70	53	25	19

*Highest titre observed

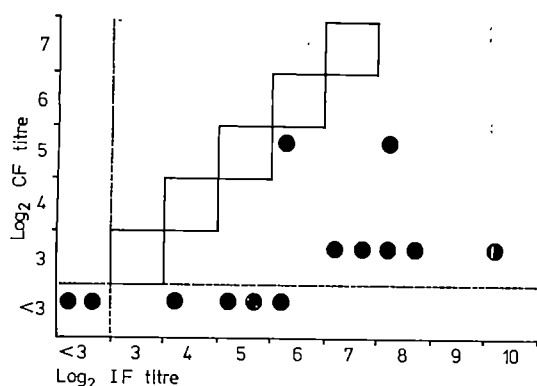


Figure Comparison of CF and IF test titres of sera from 13 *Chlamydia*-positive women

ever, *Chlamydia*-negative patients with cervicovaginal infection were significantly ($P < 0.005$) more often seropositive by IF test compared with patients with normal clinical findings (Table 2).

Four (31%) *Chlamydia*-positive patients and seven (5%) *Chlamydia*-negative patients complained of ocular and urethral symptoms. The sexual partners of three (23%) *Chlamydia*-positive women and four (3%) *Chlamydia*-negative women were known to have urethral symptoms (dysuria and urethral discharge). One of the *Chlamydia*-positive patients was using oral contraceptives, two had an IUD fitted, and two were pregnant, one in the first and the other in the second trimester.

Papanicolaou's cervicovaginal smears obtained from the *Chlamydia*-positive patients were classified as inflammatory (inflammatory cellular atypias with abundance of leucocytes) in 69% of patients compared with 42% of *Chlamydia*-negative patients. This difference was not however statistically significant ($P < 0.25$).

On colposcopy, grade I-II inflammation was observed in 11 of the *Chlamydia*-positive patients. The histological findings were: normal cervical epithelium in four patients, ectopy area with mild,

chronic infection in one, vaginitis with mild, ectocervical infection in two, and ectopy area with epidermisation in one. Colposcopic grade III inflammation was observed in two *Chlamydia*-positive patients. In the first instance the histological specimen showed a chronically infected ectopy area with squamous metaplasia and in the other a normal ectocervical epithelium with mild chronic and unspecific infection. Cytological and histological specimens were carefully screened for typical intracellular inclusions (Naib, 1970; Swanson *et al.*, 1975), but none was found.

Culture for yeast infection was positive in 14 patients, and for *N. gonorrhoeae* in five patients. Genital condylomata accuminata were diagnosed in eight patients. None of these patients had concomitant *Chlamydia*. One of the three patients with *T. vaginalis* infection and one of the four with herpes genitalis infection also excreted *Chlamydia*.

Discussion

The chlamydial isolation rate of 9% in 144 women from a general gynaecological outpatient population accords with the results of Schachter *et al.* (1975b), who found seven (8%) *Chlamydia*-positive patients out of 90 women attending a women's clinic for routine examination in San Francisco, but it is lower than the 12.7% isolation rate in women 'with symptoms' attending the same clinic (Schachter *et al.*, 1976). A higher incidence has been found in sexually more promiscuous groups, such as patients attending venereal disease clinics and contacts of men with NGU (Hobson *et al.*, 1974; Nayyar *et al.*, 1976; Richmond and Sparling, 1976; Alani *et al.*, 1977; Rees *et al.*, 1977).

The clinical picture of overt genital chlamydial infection was frequently non-specific cervicitis and cervical erosion. Similarly, Schachter *et al.* (1975a, 1976) have shown genital chlamydial infection to be associated with cervicitis and cervical erosion but not with vaginitis alone. Schachter *et al.* (1976) found an isolation rate of 34.7% among 170 women

Table 2 Comparison of clinical cervicovaginal findings and chlamydial isolation results with seropositivity

	No. of patients	Chlamydia-positive						Chlamydia-negative					
		No.		CF*		IF†		No.		CF*		IF†	
				No.	%	No.	%			No.	%	No.	%
Normal	71	3	4	1	33	1	33	68	96	6	9	6	9
Vaginitis only	22	2	9	2	100	2	100	20	91	5	25	8	40
Cervicitis/cervical erosion	51	8	16	4	50	5	67	43	84	10	23	11	26
	144	13						131					

*CF test titre $\geq 1:8$

†IF test titre $\geq 1:64$

with cervicitis. Hobson isolated *Chlamydia* in 63% of 35 women with hypertrophic cervical erosions (Hobson *et al.*, 1974). We found only 16% of the patients with cervical infection harboured *Chlamydia*, which may be because our patients were attending a gynaecological clinic rather than a clinic for the treatment of genital infections.

Ocular and urethral symptoms were significantly more common in patients with overt chlamydial infection (excretion from the cervix) than in *Chlamydia*-negative patients. Other micro-organisms examined in our study could not explain these symptoms, since yeast, condyloma accuminatum, and *N. gonorrhoeae* were not detected concomitantly with *Chlamydia*, and herpes genitalis and *T. vaginalis* were detected in only one case together with *Chlamydia*.

Much attention has recently been paid to cytology as a rapid method for diagnosing viral infections. The cytological diagnosis of herpes genitalis has been established (Naib *et al.*, 1966; Vesterinen *et al.*, 1977). Cytomegalovirus (Vesterinen *et al.*, 1975) and adenovirus (Vesterinen *et al.*, 1978) can also cause specific alterations in human cervical epithelial cells *in vitro*. Specific cytopathic alterations induced by *Chlamydia* in human cervical epithelium in clinical cases have been presented (Swanson *et al.*, 1975). In the present work special attention was paid to such alterations in cervicovaginal smear preparations and histological samples, but none was found. A non-specific inflammatory picture dominated in *Chlamydia*-positive cases indicating that cytomorphological methods are unrewarding in the diagnosis of genital chlamydial infections. Non-specific inflammatory changes have been regularly found in the cervical smears of female contacts of men with NGU (Simmons and Vosmik, 1974). Naib has recognised typical clusters of intracytoplasmic inclusions in epithelial parabasal cells in addition to an abundance of leucocytes and histiocytes in cervicovaginal smears of mothers of newborn infants with suspected chlamydial infection (Naib, 1970). However, no patient in the third trimester of pregnancy was included in our study.

Determining chlamydial group-specific antibodies by the CF test only is unrewarding in genital tract infections which tend to limit themselves to the epithelium (Grayston and Wang, 1975; Richmond and Caul, 1975). We found, however, that 54% of women with proved cervical chlamydial infection had CF antibodies, compared with 16% of *Chlamydia*-negative women. This is in accordance with the results of Schachter *et al.* (1975b), who observed that 57.5% of 40 women with overt chlamydial infection had CF antibodies.

The new modification of a single-antigen IF test applied in the present study appeared to be sensitive. *C. trachomatis* LGV-serotype L2 has been shown by IF tests to give crossreaction with antibodies to most important genital chlamydial serotypes (Oriol *et al.*, 1975). Reactive sera occurred to 85% of the *Chlamydia*-positive patients and in 53% of the *Chlamydia*-negative patients. However, only 19% of the *Chlamydia*-negative patients were seropositive at IF test titre $\geq 1:64$ as compared with 62% of the *Chlamydia*-positive patients. Similarly, Richmond and Caul (1975, 1977), using another single-antigen IF test, noted reactive sera in 88% of culture-positive and in 49% of culture-negative women attending a venereal disease clinic.

It was noteworthy that the seropositivity rate was high among *Chlamydia*-negative patients with signs of cervicovaginal infection (Table 2). This may be attributed to a past chlamydial infection or to the insensitivity of current isolation methods for *C. trachomatis*. Perhaps the serum antibodies even at high titres are not diagnostic for an active infection, since such antibody titres are also seen in isolation-negative patients (Table 1). More knowledge about the kinetics and duration of the serological response to chlamydial infection is needed.

Non-specific genital infection in women seems to be a major epidemiological problem as a reservoir of *Chlamydia*, particularly because of the difficulty in identifying chronic and latent infections.

Our study gives further evidence of the pathogenicity of *C. trachomatis* in the female genital tract. The non-specific clinical and cytomorphological picture of this infection requires that reliable isolation facilities are generally available in gynaecological practice.

We wish to express sincere thanks to Mrs Hellä Sarjakivi for her technical assistance in the CF and IF antibody assays, and to Mrs Tuula Nieminen for efficiently carrying out the secretarial work. We also thank Miss Marja-Leena Kauppinen, who kindly and reliably assisted in the collection of the specimens.

This study was aided by a grant from the Paulo Foundation and Finnish Cultural Foundation.

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Recurrent genital herpes among women : symptomatic v. asymptomatic viral shedding

MARK C. RATTRAY, LAWRENCE COREY, WILLIAM C. REEVES,
LOUIS A. VONTVER, AND KING K. HOLMES

From the Departments of Medicine and Obstetrics and Gynecology, University of Washington School of Medicine; and the Department of Epidemiology and International Health, University of Washington School of Public Health and Community Medicine, Seattle, Washington

SUMMARY To investigate whether asymptomatic shedding of herpes simplex virus occurs in women with recurrent genital herpes, six women with documented disease were followed up twice weekly with viral cultures and pelvic examination. During the study period of 1330 patient days and 452 patient visits, 26 episodes of genital herpesvirus infection were recorded. Twenty-three (88%) episodes were accompanied by signs and symptoms. Three (14%) of the culture-positive recurrences were asymptomatic. In one episode an asymptomatic lesion was noted, and in two instances viral shedding from the vulva or cervix occurred in the absence of visible herpetic lesions. Three of the six women had evidence of recurrent cervical herpesvirus infection. No relationships between menstrual cycle and sexual activity and the onset of recurrence were noted.

Introduction

Genital herpesvirus infection is the third most common sexually transmitted disease diagnosed at the Harborview Medical Center at Seattle. Previous studies on prevalence have shown that herpes simplex virus (HSV) is detected in the cervix in 0.6% of pregnant women (Bolognese *et al.*, 1976) and in 5.8% of female patients seen in a clinic for sexually transmitted diseases (STD) (Wentworth *et al.*, 1973). Seroepidemiological studies have shown that while antibody prevalence to either type 1 or type 2 virus varies with age, race, gravidity, and sexual experience antibody to HSV-2 is often found in patients without a history of clinical genital disease (Rawls *et al.*, 1970). These data suggest that genital HSV infection may initially be asymptomatic or mildly symptomatic. Cytological evidence of herpes group virus infection from cervical preparations often occurs in the absence of vulval disease (Ng *et al.*, 1970). Recent investigations have also indicated that cervical infection with HSV as demonstrated by either viral isolation or cytological changes in cervical cells was more often present in women with initial

genital infection (87%) than in women with recurrent genital disease (4%) (Adams *et al.*, 1976). In addition, 206 routine cervical cultures obtained after the first genital infection had resolved revealed no evidence of recurrent cervical HSV infection (Rattray *et al.*, 1976).

To investigate whether asymptomatic cervical excretion of herpes simplex virus occurs in women with recurrent vulval disease and to quantitate the frequency and duration of asymptomatic viral shedding we prospectively followed a small cohort of women with documented genital disease.

Materials and methods

Six women (Table 1) with previously culturally proved genital HSV infection were investigated twice weekly for a mean of 32 weeks (range 13-48). At each visit each patient was asked about her sexual and menstrual activity; a pelvic examination was performed, and any sign or symptom suggesting herpesvirus infection was recorded. Cervical cultures as well as cultures of any other suspicious genital lesion were taken routinely. Additionally, routine sweep cultures of the vulval area were taken at 42 visits from women without lesions,

To isolate HSV specimens were inoculated on to human embryonic fibroblasts overlaid with Eagle's

Address for reprints: M. C. Rattray, M.D., Department of Obstetrics and Gynaecology, University of Washington, School of Medicine RH-20, Seattle, Washington 98195, USA

Received for publication 30 June 1977

Table 1 *Details of patients*

Data	Case no.					
	1	2	3	4	5	6
Age (years)	24	22	19	22	37	27
Race	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian
Marital state	Never married	Never married	Never married	Never married	Married	Married
Socioeconomic status	Mid	Mid	Mid	Mid	Mid	Mid
Occupation	Student, free-lance artist	Medical student	Student	Student	Housewife	Administrative assistant
Parity	G ₃ P ₀	G ₃ P ₀	G ₀ P ₀	G ₀ P ₀	G ₂ P ₁	G ₀ P ₀
Contraceptive method	Oral	Oral	Oral	Oral	Tubal ligation	Oral, subsequently husband had vasectomy
Previous STD	Yeast vaginitis	Yeast vaginitis	Trichomonal vaginitis	Trichomonal and yeast vaginitis	None	None

minimum essential medium containing 2% fetal calf serum; 6.6 mmol/l sodium bicarbonate, 100 µg/ml streptomycin, 100 units/ml penicillin, 10 units/ml nystatin, and 100 µg/ml gentamicin. Tubes were incubated at 37°C. Tubes demonstrating cytopathological characteristics of HSV were passed to HeLa cells to differentiate HSV from cytomegalovirus. The initial HSV isolate from each patient was typed by the immunoperoxidase method (Benjamin, 1974). Complement fixation titres were performed as previously described (Wentworth and Alexander, 1971).

Results

During the study period of 1330 patient days and 457 patient visits, 26 recurrences of genital herpesvirus infections were detected either by the appearance of characteristic vulval lesions or by a positive viral culture in the absence of lesions (Table 2). Of the 26 recurrences, 23 (88%) were accompanied by clinical signs and symptoms. Sixteen of the symptomatic recurrences yielded positive cultures of vulval lesions and negative cervical cultures. In two (8%) of the symptomatic recurrences, HSV was isolated from vulval lesions and the cervix. In five other

recurrences, the patients presented with symptoms and lesions characteristic of genital herpetic infection, but the cultures were negative. The mean duration from onset of symptoms to viral sampling was 3.3 days in these culture-negative episodes compared with 1.3 days for those that were culture-positive [$P < 0.05$ (t test)]. Thus, the negative cultures were probably due to the later sampling of the lesions and the short duration of viral shedding seen with recurrent genital disease.

Asymptomatic viral shedding was recorded in two of the six patients in the study and accounted for 12% of the HSV recurrences (14% of the culture-positive episodes). Asymptomatic vulval shedding was found twice during the course of the investigation. On one occasion the patient had no symptoms of vulval irritation, but a small 2 × 3 mm wet, vulval ulcer, unnoticed by the patient, was found on examination; HSV was grown from the lesion. It was then decided to sample routinely the vulval area of all patients at all visits in the future with a cotton swab and to submit these specimens for viral culture. One of these 42 subsequent vulval sweep cultures grew herpes simplex virus. The patient was without signs or symptoms

Table 2 *Frequency of asymptomatic v. symptomatic recurrences among women with genital herpes*

	Case no.						Total
	1	2	3	4	5	6	
No. of weeks followed up	47	46	31	29	24	13	190
Total recurrences	5	7	10	2	2	0	26
Recurrences with lesions and symptoms							
Vulva (culture-positive)	4	3	7	2	0	0	16
Vulva (culture-negative)	1	3	0	0	1	0	5
Vulva and cervix (culture-positive)	0	1	1	0	0	0	2
Recurrences without symptoms (culture-positive)							
Vulva with lesion	0	0	1	0	0	0	1
Vulva without lesions	0	0	1	0	0	0	1
Cervix without lesions	0	0	0	0	1	0	1
Frequency of recurrences (per 100 patient days)	1.52	2.17	4.61	0.99	1.19	0	1.95 (mean)
Months since primary infection	9	11	1	10	24	72	
HSV type	2	2	2	2	2	1	

at the time of sampling. A vulval sweep culture at the next visit, two days after the positive isolation, did not grow HSV.

Asymptomatic cervical excretion was found once during the course of the study. During the period between clinical recurrences 429 cervical cultures were taken when patients were without signs or symptoms. Of these only one (0.2%) was positive for HSV. The duration of viral shedding was short; viral cultures were negative at the next visit three days later. Neither signs of vulval or cervical lesions, nor symptoms of increased vaginal discharge or irritation, was noted during the period of cervical viral shedding.

Among the cohort the mean number of recurrences per patient was 4.3 (range 0-10). The frequency of recurrence ranged from 0.99 to 4.6 per 100 patient days with a mean of one recurrence every 53 days. Among those women studied within one year of their primary genital HSV infection, the frequency of recurrence was 2.2 per 100 patient days compared with 0.77 recurrences per 100 patient days in women with a history of HSV for longer than 24 months before being studied. No relationship between the frequency of recurrence and titre of complement fixing (CF) antibody to HSV was noted, and the patient (Case 3: Table 1) with the highest CF antibody titre (1:128) experienced the greatest number of recurrences. In addition, a rise or fall of more than 1 tube dilution in CF antibody titre in serum obtained at the beginning and end of the study did not occur in any patient. It is of interest that the only woman who experienced no recurrence was the only one initially infected with HSV-1. Although she was followed up twice weekly for only 13 weeks, less frequent follow-up revealed no symptomatic recurrences during the subsequent eight months.

The mean number of lesions during recurrences was 2.7 (range 1-12) and the mean duration from

onset of symptoms to healing was six days (range 2-10). During symptomatic recurrences the duration of viral shedding, defined as the time from onset of lesions until the last day HSV was isolated from the lesions, was 1.9 days. As patients were seen at 3-4 day intervals, this figure represents a minimal estimate of the duration of viral shedding. Pain was present for an average of 5.2 days and itching 4.3 days. An increase in vaginal discharge was noted in 42% of the symptomatic recurrences, dysuria in 13%, and constitutional symptoms of malaise and fever were present in only one (4%) of the recurrences.

Before entering the study, four of the six women felt that their recurrences were precipitated by the onset of menses. When the date of onset of recurrences was analysed by time of menstrual cycle (Figure), no discernible relationship between menstrual period and onset of recurrent genital infection was present. Sixty-five per cent of recurrences began during midcycle, 23% during menses, and 11% in the five days preceding menses. It should be noted, however, that 24 of the 26 recurrences occurred in women taking oral contraceptives, and it is not known whether this mode of contraception affects the recurrence pattern of genital herpesvirus infection.

In addition, there was no obvious relationship between sexual activity and recurrent herpes virus infection in these women. All the women were sexually active. The mean number of days between last sexual contact and positive herpes cultures was 2.0 days compared with 2.7 days between last sexual contact and those visits not associated with recurrence ($P > 0.05$ (t test)).

Discussion

In this study of women with recurrent genital herpes asymptomatic viral shedding accounted for 14% of

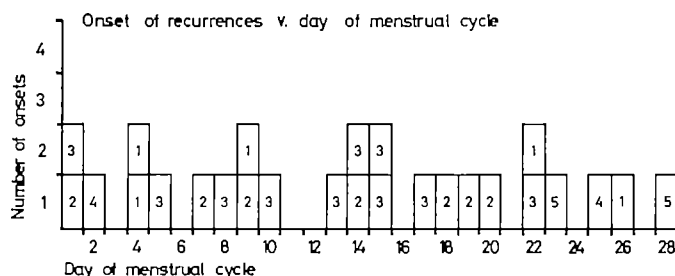


Figure Onset of genital herpesvirus recurrence by day of menstrual cycle among women with recurrent disease

the HSV culture-positive recurrences. In women with recurrent disease, both asymptomatic herpetic lesions and transient shedding of HSV from cervix or vulva without obvious herpetic lesions were documented. These data appear analogous to those of Douglas and Couch (1970), who reported that 2.2% of saliva specimens taken from asymptomatic patients with serological evidence of infection were positive for HSV (Douglas and Couch, 1970). Thus studies of the natural history and prevention of recurrent herpes should examine both symptomatic and asymptomatic disease.

The role that asymptomatic viral shedding may play in the transmission of genital HSV infections is unknown. Many patients presenting with initial genital HSV infections have sexual partners without recent signs or symptoms of infection. These contacts usually have serum antibody to HSV, but no clinically detectable HSV infections are present when they are examined. The data from this study suggest that these sexual partners may have had unnoticed lesions or transient shedding of HSV from the cervix or other sites without lesions.

Three of the six women with recurrent vulval disease shed HSV from the cervix at some time during the course of the investigation. It is possible that some women with recurrent genital herpes do not develop cervical recurrences and may have a risk of cervical carcinoma which differs from the risk seen in women with recurrent cervical shedding (Naib *et al.*, 1966; Rawls *et al.*, 1976).

The frequency of recurrence of genital disease was greater in women who had a history of genital herpes for less than 12 months before the beginning of the study (2.22 recurrences per 100 patient days) than in those with genital HSV of more than two years' duration (0.77 recurrences per 100 patient days). Further analyses of the frequency of recurrence in a larger number of intensively studied patients are needed to determine whether there is a decrease in the rate of shedding of HSV or a change with time in the pattern of symptoms associated with recurrence.

While all six women claimed that their herpetic recurrences were related to either menses or sexual activity, no discernible patterns between menses or sexual intercourse and the onset of recurrences were found. Fifteen recurrences began during days 7-21 of the cycle, and 12 recurrences began in the two weeks before and including the menses.

In previous studies the frequency of recurrence of genital herpes was found to be greater in men than women (Corey *et al.*, 1976; Vontver *et al.*, 1978). Whether this increased frequency of recurrence in men is due to a difference in the rate of viral shedding of HSV in men compared with women or if self-detection of trivial herpetic lesions is easier in men than in women remains unknown. Similar studies are needed in men to investigate the anatomical site, frequency, duration, and potential transmissibility of asymptomatic HSV shedding.

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The value of a psychiatric service within the venereal disease clinic

S. BHANJI* AND J. D. H. MAHONY†

*From the *University of Exeter Postgraduate Medical Institute and the †Alexanda Clinic, St Giles Hospital, London*

SUMMARY A significant proportion of attenders at venereal diseases clinics can be regarded as potential psychiatric patients. However, there have been few reports on the value of readily available psychiatric advice at such clinics. The findings during a three-month period when a psychiatrist attended a venereal disease clinic are described. Twenty-two patients were referred for psychiatric assessment. Of the 20 who kept their appointment, 18 were diagnosed as mentally ill or as having a personality disorder. Of these two were already under psychiatric care, but 11 had never previously sought psychiatric assistance. The major sources of common venereological and psychiatric concern appeared to be among the promiscuous and the hypochondriacal patients. The former consisted of persons with personality disorders who were thought unlikely to respond to psychiatric treatment; but some of the latter improved after treatment with pimozide. Some patients defaulted and not all who persevered were helped. Nevertheless the results of a questionnaire showed that the staff considered there was an important place for a psychiatrist within their clinic.

Introduction

Several authors have reported marked psychiatric morbidity and psychosocial distress among patients attending clinics for sexually transmitted diseases. As yet, few clinics have a psychiatrist in regular attendance. We recently carried out a study to determine whether there was a useful role for a psychiatrist in a clinic and to delineate common areas of concern.

Materials and method

The Alexanda Clinic serves an area in south-east London in which about 90% of the population belong to social classes III, IV, and V, and 10% are from overseas, mainly the West Indies. The clinic is staffed by four doctors, six nurses, and two social health workers (SHWs). During the three months beginning March 1977 a psychiatrist (SB) attended twice weekly to assess and, if possible, treat any patient whom any member of the clinic staff considered was psychologically disturbed. The views of the clinic staff were ascertained by a questionnaire at the end of the study.

Results

During the period of the study 368 male patients and 358 female patients attended with new episodes of infection. Most had genital infections other than syphilis or gonorrhoea, but approximately 20% had no evidence of genital infection. Fifteen men and seven women were referred for a psychiatric opinion. The primary reasons for referral could be divided into four categories (Table 1): promiscuity associated with psychological disorder (Group 1), psychogenic physical complaints which were not relieved after reassurance (Group 2), psychogenic sexual dysfunction (Group 3), and pathological depression (Group 4).

The 20 patients who kept their appointments are shown in Table 1 according to the primary reason for referral. Their venereological diagnoses are shown in Table 2. Four patients had never contracted venereal disease, but eight had been infected at least twice. Two of the patients (Cases 8 and 19) were already receiving psychiatric treatment elsewhere, and five had previously seen psychiatrists but not necessarily for their current problems.

Table 1 shows that the presence of psychiatric or psychological disorder was confirmed in all but two patients and that the primary psychiatric conditions were personality disorder (five patients), depressive

Address for reprints: S. Bhanji, University of Exeter, Postgraduate Medical Institute, Barrack Road, Exeter EX2 5DW

Received for publication 30 November 1977

Table 1 Patients seen by psychiatrist

Case No.	Sex	Age	Origin	Diagnosis	Times seen
<i>Group 1: Promiscuity</i>					
1*	M	40	UK	Personality disorder, homosexuality	1
2*	M	28	UK	Personality disorder	1
3*	M	22	West Indies	Personality disorder	1
4	M	33	UK	Personality disorder, homosexuality, depression	1
5	F	17	UK†	Adolescent behaviour disorder	2
6*	F	20	UK	No psychiatric disorder	1
<i>Group 2: Psychogenic complaints referable to somatic disorder</i>					
7*	M	21	Iran	Depression with hypochondriasis (venereophobia)	1
8	F	46	Eire	Depression with hypochondriasis (venereophobia)	1
9*	M	39	Nigeria	Primary hypochondriasis (venereophobia)	5
10*	M	31	UK	Primary hypochondriasis (venereophobia)	2
11	F	20	UK	Obsessional neurosis with hypochondriasis (venereophobia)	3
12*	M	38	Nigeria	Primary hypochondriasis (venereophobia)	4
13	M	22	West Indies	Primary hypochondriasis (dermatozoiasis)	4
<i>Group 3: Psychogenic sexual dysfunction</i>					
14*	M	27	West Indies	Psychogenic impotence	2
15*	M	29	UK	Psychogenic loss of libido	1
16*	M	38	West Indies	No dysfunction	1
<i>Group 4: Depression</i>					
17	M	66	Poland	Depression	1
18*	F	56	Eire	Depression (grief reaction)	1
19	F	32	UK	Personality disorder, mixed neurotic state	1
20*	M	38	Holland	Depression	2

*Patients with no previous psychiatric contact

†Of West Indian parentage

Table 2 Venereological diagnoses at time of psychiatric assessment

Diagnosis	Case No.
Syphilis	4
Gonorrhoea	1, 2, 3, 5, 16, 19
Other genital infections	6, 14, 15, 18, 20
No genital infection	7, 8, 9, 10, 11, 12, 13, 17

illness (5), primary hypochondriasis (4), psychogenic sexual dysfunction (2), obsessional neurosis (1), and adolescent behaviour disorder (1). Primary hypochondriasis was diagnosed if the patient's conviction of illness appeared to be circumscribed and not derived from underlying mental illness.

The extent of social problems can be assessed from the finding that three of the 10 patients who had married were divorced or separated, 10 were unemployed, three drank heavily (although without signs of physical dependency on alcohol), and three admitted to involvement with the police. All those referred because of promiscuity were venereological recidivists, as were four of the five with personality disorders. There were no racial differences in venereological diagnosis, in reason for referral, or in psychiatric diagnosis.

Of the 12 patients who were seen only once two had no evidence of psychiatric illness, four were thought unlikely to benefit from treatment (Cases 1, 3, 4, and 17), two were already under psychiatric care (Cases 8 and 19), one had gone abroad (Case 7), and three declined treatment (Cases 2, 15, and

18). Of the patients who accepted treatment five had not previously sought psychiatric advice.

The outcome of treatment varied. One patient (Case 20) was referred elsewhere, and one (Case 5), whose condition had improved with treatment, was advised to see the SHW for further counselling. The patients with hypochondriacal complaints were prescribed pimozide (Riding and Munro, 1975). The condition of three patients (Cases 9, 10, and 11) improved. The male patient with impotence was advised to attend with his wife but failed to do so. Finally, two of those seen only once (Cases 2 and 4) telephoned to report improvement.

At the end of the study a questionnaire was completed anonymously by the clinic staff. Ten reported an involvement in referring patients, and seven stated that this was helpful. Ten of the staff considered that regular psychiatric attendance in the future would be desirable.

Discussion

Surveys carried out using standardised screening procedures have shown much psychological abnormality among patients with venereal disease (Wells, 1970; Mayou, 1975). Mayou (1975) found that 20% of patients could be regarded as potential psychiatric cases, and Pedder and Goldberg (1970) reported an even higher prevalence. Most investigations of individual psychopathology have been confined to patients presenting with certain symptoms, such as syphilophobia (MacAlpine, 1957) and

fear of venereal disease (Kite and Grimble, 1963), or with deviant or unconventional life-styles—for example male clients of prostitutes (Gibbens and Silberman, 1960), homosexuals (Fluker, 1966), and drug addicts (Linken, 1968). A recent exception is the report by Pedder (1970) of 40 unselected psychiatric patients referred during a 16-month period. The patients represented only 0.3% of the total new attendances.

Our patients compared with those of Pedder (1970) regarding sex and age distribution, marital state, and venereological diagnosis, but more of them were born outside the United Kingdom. There were more patients with hypochondriacal complaints in our study, but there were relatively fewer with personality disorders. In both studies several patients were seen on only one occasion, and a significant proportion of those offered treatment defaulted. Pedder (1970) did not state how many of his patients were already under psychiatric care, but he gives this as a reason for some being seen only once.

Most of the clinic staff welcomed the prospect of having readily available psychiatric expertise. At the end of the study this attitude had not altered overall. This is perhaps surprising as few patients showed any evidence of having been helped. However, it is possible that the benefit of easy access to psychiatric advice outweighed the disappointing outcome in certain instances.

The main areas of common venereological and psychiatric concern among the patients were promiscuity and hypochondriasis. Unfortunately the promiscuous group consisted largely of patients who were considered unlikely to benefit from psychiatric treatment. Venereophobia was the commonest of the hypochondriacal complaints. Controversy exists, as with hypochondriasis in general (Kenyon, 1976), over whether venereophobia occurs solely as a symptom of underlying mental illness or whether it can exist as an independent entity. MacAlpine (1957) regarded syphilophobia as a psychiatric symptom which could accompany or precede all stages of mental illness. Similarly, Kite and Grimble (1963) and Mayou (1975) found that venereophobia was associated with a variety of different psychiatric syndromes. Pedder (1970), by contrast, described three patients who showed no evidence of underlying psychiatric disorder. Our data likewise suggest that venereophobia can present in isolation. The use of pimozide in this

condition merits further study. Finally, it appears that a small but significant number of patients with psychosexual dysfunction may present to the venereologist.

In conclusion, our experience accords with the view that a number of patients attending clinics for venereal diseases show sufficient psychopathology to justify specialist assessment. Furthermore, in some instances the psychological and genital symptoms appear to be interrelated. This finding, together with the fact that most of our psychologically disturbed patients were not under psychiatric care, suggests that there is an important place for the psychiatrist within the venereology clinic. However, not all the patients referred were willing to accept psychiatric help, and of those who were not all benefited.

This work was carried out while one of us (SB) was Honorary Senior Registrar to the Academic Department of Psychological Medicine, King's College Hospital, London SE5 9RS. Our thanks are owed to Professor R. H. Cawley for his encouragement and comments during the preparation of this paper. The demographic data on the Alexandra Clinic's catchment area were kindly provided by the MRC Social Psychiatry Unit, Institute of Psychiatry, London SE5 8AF.

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Venereal disease education in West Virginia, USA

JOSEPH C. FRANZ AND ROLAND J. WEISSER JR

From the Committee for Venereal Disease Education, West Virginia University, Morgantown, West Virginia, USA

SUMMARY A venereal disease educational programme entitled Venereal Disease Education in West Virginia was constructed. It consisted of a preliminary test to assess the level of general knowledge of venereal diseases, a value survey list, a lecture illustrated by colour slides, and a final test. The programme was presented to 3210 students in Northcentral West Virginia who ranged in age from 12 to 20 years (7th to 12th grade). The mean score in the preliminary test was 7.97 (53%) correct answers out of 15, and the mean score for the final test was 11.55 (77%) correct answers out of 15. The ability of students to retain the information given to them was measured by testing the same class of 53 students one year later. These students obtained a mean score of 11.41 (76%) correct answers out of 15 in the later test which demonstrates that the students had retained much of the information.

Introduction

The assignment of responsibility for venereal disease education in schools is often discussed. Until October 1975, venereal disease education in schools in West Virginia had been largely the responsibility of the state Health Department, but limited manpower and lack of money had prevented a major attack being made. The Health Department had mainly concentrated on distributing pamphlets but occasionally field health officers had run courses on venereal diseases. There had also been sponsored roadside advertisements, and announcements for television and radio. In some areas local physicians had given their time to schools while in others the topic was lightly covered by various courses on health education, physical education, or human sexuality. These efforts could be described as sporadic.

This paper describes an educational programme developed and tested during the last two years by the Committee for Venereal Disease Education of the West Virginia University Medical Center. The programme is unique in at least two respects. Firstly, the manpower is derived from energetic students studying medicine, pharmacy, or nursing at West Virginia University Medical Center. Secondly, it integrates tests, with lectures and discussion groups so that the 'package' provides an educational

'learning experience' rather than just a didactic presentation, and some measure of its effectiveness is available.

Methods

The venereal disease educational programme comprised a preliminary test on venereal disease in general, a value survey list, a lecture illustrated with colour slides, and a final test. It was reviewed and approved by state and local Health Department officials and by the Public School Superintendents and Boards of Education of five counties in Northcentral West Virginia.

The study was aimed at 9th grade students who were selected for two reasons. Work done by an epidemiologist from a local state Health Department showed that between the 7th and 9th grade there is a greater sexual awareness by both girls and boys (Nuzum, personal communication). Secondly, the 9th grade classes in most schools are smaller and therefore more suitable for presentations by groups from outside. However, because there were many requests for the programme and because of the need for such education, other groups of students were subsequently included.

The principal of each school in the five counties that had 9th grade students was sent a letter which informed him of the high incidence of venereal disease and sought permission to present the programme to his students. Parental approval was obtained by giving each student a permission slip which had to be returned to the principal only if the student was *not* to take part.

Address for reprints: J. C. Franz MD, Committee for VD Education, Box 1161, Medical Center, West Virginia University, Morgantown, West Virginia 26506, USA

Received for publication 30 June 1977

Approximately 14 days before a presentation, students were asked 15 preliminary questions to test their general knowledge of venereal disease. On the day of the presentation they were first given a list of five 'values' (that is, 'a comfortable life,' 'having children,' 'having a sound mind' etc.) each of which related to health. The students were then asked to rate these values in order of priority (1 being the most important, 5 being the least important). The students were asked if they could identify ways in which venereal diseases might affect any of these values.

The illustrated lecture dealt primarily with syphilis and gonorrhoea. The programme began by defining venereal disease and giving some general information. It then changed the emphasis to cover more specifically the incidence, causes, symptoms, and treatment. This first part of the programme lasted approximately 30 minutes. Then the class was divided into smaller groups of 6-10 students, with a moderator from the Committee for Venereal Disease Education allocated to each group who would answer questions or talk individually to group members. He also conducted 'role playing' with the students by giving them hypothetical situations—such as pretending the students had just arrived at the clinic—to see how they would respond. He then provided a model for handling

this situation. The moderator might also ask the students about various topics on venereal diseases.

After this 15-minute discussion, there was a formal summing up in which the entire class took part. This five-minute segment reviewed the cardinal signs and symptoms of syphilis and gonorrhoea and attempted to dispel myths about venereal disease. Finally, the programme gave the addresses of local facilities for diagnosing and treating venereal diseases and emphasised the confidential nature of such services. The importance of contact tracing was also explained. The class was then taken on a pictorial tour of a local clinic. Pamphlets summarising the programme and giving addresses of local treatment centres were then distributed. Two weeks after the presentation, a second test (identical with the preliminary one) was administered to the group by the class instructors; this was returned to the Committee for evaluation.

Results

During the last two years, 85 separate presentations have been given to 3210 students who ranged from 12 to 20 years of age (7th to 12th grade). The students' knowledge of venereal disease was evaluated by using a selection of 15 multiple-choice questions for the preliminary and final tests. The

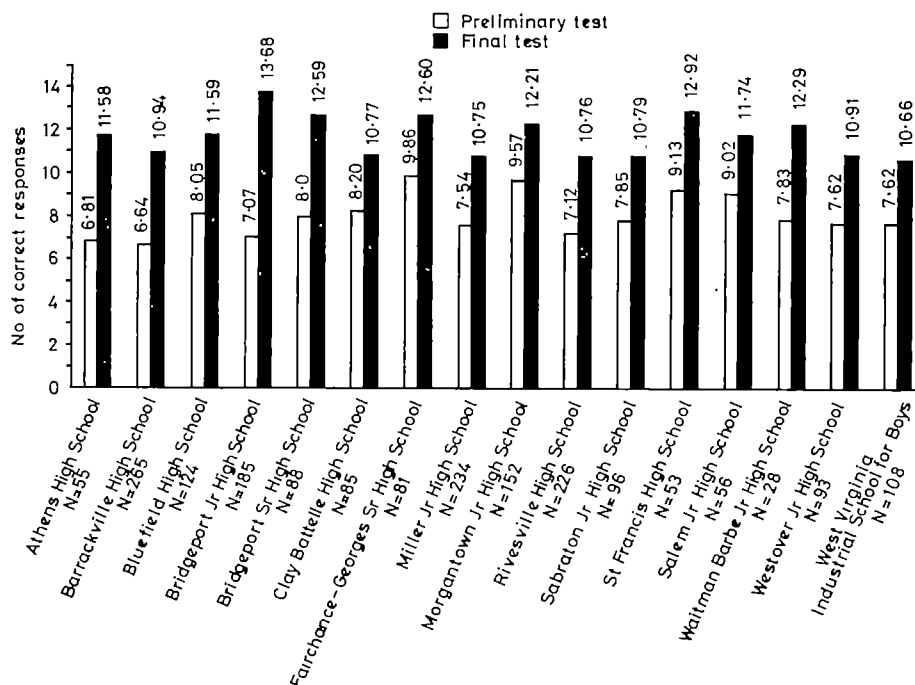


Fig. 1 Mean preliminary test and final test results.

mean preliminary test score was 7.97 (53%) correct answers out of 15, and the mean final test score was 11.55 (77%) correct answers out of 15. Figure 1 shows some of the schools visited. The highest mean preliminary test score (9.86 correct answers out of 15) was obtained by 10th, 11th, and 12th graders at Fairchance-Georges Senior High School where the students had been given an introductory course earlier in the year. The highest mean final test score was obtained by 9th graders at Bridgeport Junior High School where the instructor had included our programme as part of a teaching course on venereal disease. These rough scores suggest that all the students had better test scores after the presentations but they do not reflect what the students had learned.

To obtain a better evaluation of what the students had learned, the 15 questions in the preliminary and final tests were divided into four general categories and a question-by-question analysis was performed by comparing the percentage of students answering each question correctly in the preliminary

test and the final test. The four categories consisted of:

1. Venereal disease in general
2. How venereal disease is contracted
3. Syphilis
4. Gonorrhoea.

There were three questions on venereal disease in general (Fig. 2). The most remarkable result was that to question number 14 which measured the student's knowledge of whether or not his parents had to be notified if he was treated for a venereal disease. In the preliminary test, only 47% of the students knew that by law no parent could be notified without their consent. After the presentation, 89% of the students knew this to be true.

Four questions dealt with the ways in which venereal disease is contracted (Fig. 3). These tested the students to see if they knew that: (1) it is transmitted by 'skin to skin' contact; (2) it can be transmitted by either an infected man or woman; (3) syphilis and gonorrhoea can be present simul-

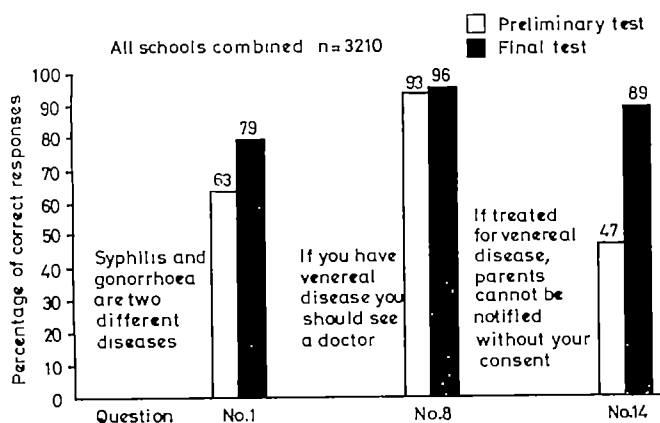


Fig. 2 Questions dealing with venereal disease in general.

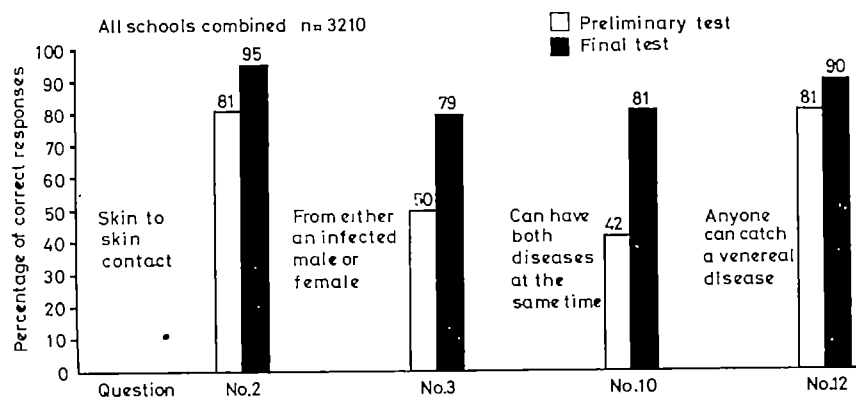


Fig. 3 Questions dealing with how venereal disease is contracted.

taneously; (4) no one is immune to venereal disease. In the preliminary test, only 42% of the students knew that gonorrhoea and syphilis could occur simultaneously but by the final test 81% knew this was correct.

Originally five questions dealt with syphilis. However one of these was found to confuse the students and so it was not used in the results for 1975-76 (Fig. 4a). It dealt with the location of the first sign of syphilis. In 1976-77 the question was changed so the answers to question number 9 were from a smaller number of students (Fig. 4b). The other questions tested the students to see if they knew: (1) the first sign of primary syphilis; (2) that treatment is still required even though symptoms of syphilis have resolved; (3) that an unborn child can be affected by syphilis; and (4) untreated syphilis can lead to insanity. In the preliminary test, only 66% of the students knew that treatment for syphilis was required after the

first stage had resolved; 90% of the students knew this after the presentation.

The last group of three questions dealt with gonorrhoea (Fig. 5). These tested to see if the students: (1) could identify the disease by its symptoms; (2) understood that untreated gonorrhoea could lead to sterility; or (3) knew that gonorrhoea was the most widespread communicable disease. The most striking result was the fact that in the preliminary test only 22% of the students knew gonorrhoea was the most widespread communicable disease. After the presentation, 66% of the students understood its severity.

To evaluate what knowledge had been retained, 53 students who had attended the programme as 9th graders were tested again a year later without being told beforehand. Originally, their mean score in the preliminary test had been 9.34 (62%) correct answers out of 15 and their mean final test score 12.63 (84%) correct out of 15. One year later,

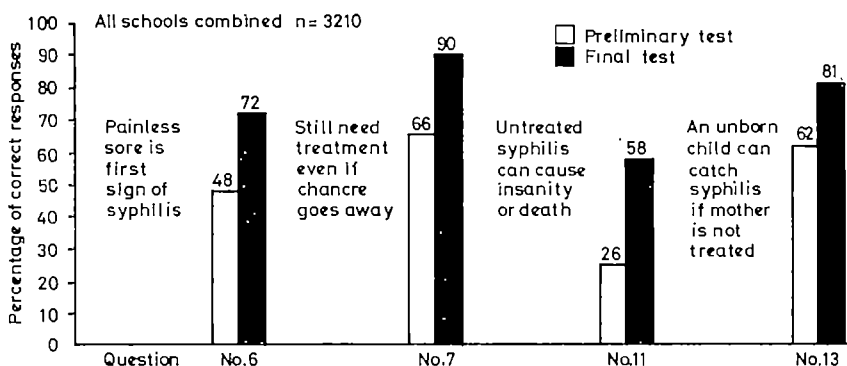


Fig. 4a Questions dealing with syphilis.

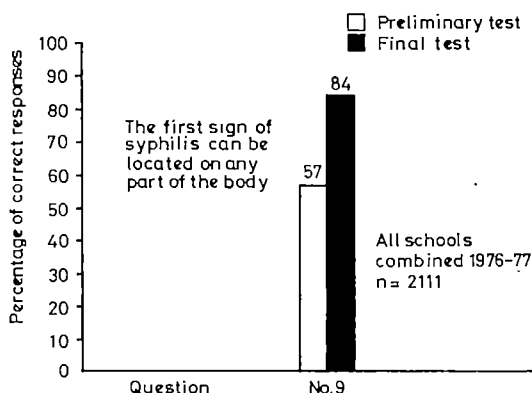


Fig. 4b Questions dealing with syphilis.

the students' mean score in the final test was 11.41 (76%) correct out of 15 (Fig. 6). Again a large percentage of the students answered the questions dealing with the signs and symptoms of syphilis and gonorrhoea correctly. The students found the questions about the results of untreated venereal disease difficult—a point that is often not stressed. Overall, 75% or more of the students answered 10 of the 15 questions correctly and 13 of the 15 questions were answered correctly by 61% or more of the students, one year after the programme.

Discussion

It is generally accepted that education influences disease prevalence to some extent (Simmons, 1971). It is also known that making students take part in

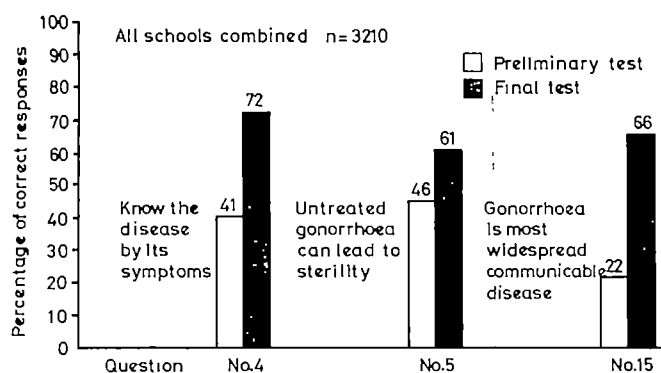


Fig. 5 Questions dealing with gonorrhoea.

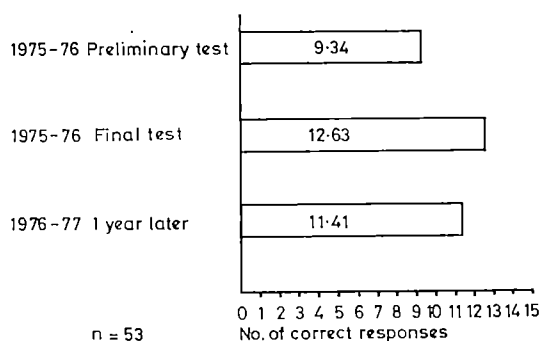


Fig. 6 Mean preliminary test and final test results compared with the results to the same tests one year later (at one school).

performance tests, discussions, and self improvement analysis is more effective than merely providing information in the form of pamphlets (Snegroff, 1975). In addition to providing the right kind of venereal disease education, a better understanding of the attitudes of young people towards sexual matters and a more open attitude towards the topic of venereal disease are needed.

The facts about syphilis and gonorrhoea, as well as other sexually transmitted diseases, are relatively straightforward and can easily be learned and retained, as shown by our study. Therefore, besides providing the facts, venereal disease education must give the following:

1. A feeling of trust when approaching a health professional for treatment
2. An understanding of the importance of contact tracing and how it works
3. An understanding of the function of a clinic
4. The knowledge that venereal diseases are ordinary conditions that doctors see and treat every day.

These extra necessities can be met by the use of small discussion groups as illustrated in this paper. It is often stated that if a teenager himself seeks treatment for a venereal disease, it will probably be the first time that he has sought medical help without his parents. The impression the teenager receives from the moderator from the Committee for Venereal Disease Education will often influence his opinion of health professionals and his trust in them. Small group sessions should be carried out only by mature and responsible individuals who understand the emotions and needs of the teenager. Until venereal disease can be accepted as just an illness and without the present stigma, we will continue to have a serious problem. Public awareness may prove to be a major factor contributing to the eventual decline in the prevalence of venereal disease.

We wish to acknowledge the assistance of members of the Committee for Venereal Disease Education of the West Virginia University Medical Center and to thank the West Virginia State Department of Health and State Department of Education for their assistance in the development and evaluation of our venereal disease education programme. We are grateful to the Bureau of Health Education of the Center for Disease Control for their financial support over the last two years and the assistance of the American Medical Student Association for administration of these funds.

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Urinary tract infection in a clinic for sexually transmitted diseases

M. G. MEAD AND R. N. GRÜNEBERG

*From the Departments of Microbiology and Genitourinary Medicine,
University College Hospital, London WC1*

SUMMARY A study of the prevalence of urinary tract infection (UTI) in 430 women attending a clinic for sexually transmitted diseases and its relationship to sexual activity is presented. UTI, usually asymptomatic, was found to be slightly more common in women attending the clinic than in the general population, its prevalence being 4.9%. UTI was more commonly found in patients who gave a history of recent sexual intercourse, which suggests that recent coitus was a factor in the development of significant bacteriuria. The likelihood of finding significant bacteriuria was not related to the number of sexual partners in the previous year. Trichomoniasis was more common in the small group of patients with UTI than in other women. The most common causative organism of UTI was *Escherichia coli*, and the isolates were usually sensitive to ampicillin, sulphonamides, trimethoprim, and nitrofurantoin.

Introduction

Urinary tract infection (UTI) is a very common problem in clinical practice, yet it is still little understood. It is diagnosed by the finding of significant bacteriuria, a pure growth of more than 100 000 organisms/ml of urine from two consecutive specimens. The terms 'urinary infection' and 'significant bacteriuria' are used synonymously here, as in most recent publications. Significant bacteriuria may or may not be associated with symptoms such as frequency and dysuria. Asymptomatic bacteriuria is important in pregnancy, when it may be associated with pyelonephritis in the later months (Kass, 1960).

The prevalence of significant bacteriuria varies with both age and sex. It is rare in adult males. In females it varies from about 1-2% in five-year-old schoolgirls (Savage and Wilson, 1973) to 3-5% in young, sexually active women. The greater prevalence of UTI in women during the child-bearing years than before the menarche has led to the suggestion that coitus may play a role in its pathogenesis (Grüneberg, 1970). Support for this suggestion has come from various studies. Kunin (1970), in a

long-term study of recurrent bacteriuria in school-girls, found that sexual intercourse was related to the acquisition of bacteriuria and of symptomatic infections. Sexual intercourse is indeed often recognised by patients as a precipitating factor in the development of a urinary tract infection (Grüneberg, 1970).

Kunin and McCormack (1968) compared the prevalence of significant bacteriuria in 2882 white nuns with that in 2302 white, working women (most of whom were married). They found that in the age group 15-34 years significant bacteriuria was 12.8 times more common in working women than in nuns, whereas in the age group 55 and over it was only 1.4 times as common. Only two (0.3%) of 698 white nuns aged 25-34 years had significant bacteriuria. In view of the possible association with sexual intercourse it would be of interest to know its prevalence in a clinic for sexually transmitted diseases (STD). The present study had three aims: firstly, to determine the prevalence of urinary tract infection in female patients attending a clinic for STD; secondly, to investigate the relationship, if any, of sexual activity to the finding of significant bacteriuria; and, thirdly, to ascertain whether there was a relationship between the presence of urinary tract infection and the presence of gonorrhoea, trichomoniasis, or candidosis.

Address for reprints: M. G. Mead, Department of Microbiology,
University College Hospital, Gower Street, London WC1E 6AU

Received for publication 15 December 1977

Materials and methods

STUDY POPULATION

The series comprised 430 consecutive, unselected female patients attending the Department of Genitourinary Medicine, University College Hospital. Fewer than 5% of them had been seen before in the clinic and none within the previous six months. Each patient was asked her age, the number of sexual partners she had had in the last year, the date of the last sexual intercourse, and whether she had taken antibiotics during the last three months.

CLINICAL PROCEDURES

Patients were examined in the lithotomy position and specimens taken from the vagina, cervix, and urethra for microscopical examination and culture for *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, and *Candida albicans*. After examination each patient provided a midstream urine specimen (MSU), which was immediately stored at 4°C. Each patient with UTI was treated with an appropriate antibiotic and followed up to establish that the infection had been eradicated.

LABORATORY PROCEDURES

Urethral and cervical smears were Gram-stained for intracellular Gram-negative diplococci; cultures for *N. gonorrhoeae* were inoculated on to VCNT medium (Phillips *et al.*, 1972). Vaginal specimens were suspended in saline and examined microscopically for trichomonads and for yeast cells and hyphae. Culture for *C. albicans* was on Sabouraud's medium. *T. vaginalis* was not cultured.

The MSU specimen was screened for bacteriuria within six hours by the method of Leigh and Williams (1964) on cystine lactose electrolyte-deficient (CLED) medium. Each specimen was plated in duplicate. After overnight incubation those with over 30 colonies of pure growth per inoculum area were examined by routine bacteriological methods to confirm the presence of bacteriuria greater than 100 000/ml and to identify and determine the sensitivity of the bacterium. If a positive result was obtained a second specimen was requested at the patient's follow-up visit a few days later and was treated in the same way.

Results

The mean age of the 430 patients was 25.4 years and the median age was 24 years; over 75% of the patients were aged between 18 and 30 years.

The mean number of sexual partners for each of the patients in the previous year was two. Twelve patients said they had had no sexual partners in the previous year, 212 had had one, 116 had had two, 40 had had three, 24 had had four, and 26 patients had had five or more.

When the MSUs from the 430 patients were screened, 23 were found to yield a pure growth of greater than 100 000 bacteria/ml on the first occasion; of these patients, 10 again had a positive result on a second consecutive specimen a few days later while two had a negative result on the second occasion and were excluded from the study. The remaining 11 patients had a positive culture on the first occasion but did not have a further MSU examination on a follow-up visit; of these, six failed to attend for follow-up, and five had been treated with antibiotics at the first visit (two because of dysuria and frequency). All these 11 patients have been included in the study as having UTI despite the degree of uncertainty regarding diagnosis, as a single specimen is only 80% reliable as an indicator of infection (Kass, 1956). Thus, 21 out of 430 women had UTI, giving a prevalence of 4.9%—a similar result to that found in patients attending antenatal clinics (Grüneberg *et al.*, 1969).

The relationship of the number of sexual partners to the diagnosis of UTI, gonorrhoea, candidosis, and trichomoniasis is shown in Table 1. Gonorrhoea was present in 30 (7%) of the 430 patients, candidosis was present in 99 (23.0%), and trichomoniasis was present in 35 (8.1%). Of the 30 patients with gonorrhoea, seven had an associated trichomoniasis but none had UTI. Of the 99 patients with candidosis, five (5.1%) had UTI; whereas of the 35 patients with trichomoniasis, three (8.6%) had UTI. The overall prevalence of UTI in the 430 patients was 4.9%; thus UTI was more common in those patients with trichomoniasis.

The interval between the last coitus and the attendance of the patient at the clinic is shown in Table 2. Four of the patients had urinary symptoms,

Table 1 Diagnosis in relation to number of sexual partners in previous year

No. of sexual partners	No. of patients	Diagnosis*									
		UTI		Gonorrhoea		Candidosis		Trichomoniasis		None of these	
		No.	%	No.	%	No.	%	No.	%	No.	%
0-2	340	16	4.7	18	5.3	83	24.4	26	7.6	220	64.7
3 or more	90	5	5.6	12	13.3	16	17.8	9	10.0	57	63.3
Total	430	21	4.9	30	7.0	99	23.0	35	8.1	277	64.4

*Patients with more than one infection appear under each diagnosis

Table 2 Interval between most recent coitus and attendance at the clinic

Interval (days)	Patients	UTI	
		No.	%
1 or less	45	7*	15.6
2-4	97	4*	4.1
5-14	148	5	3.4
15-30	68	3	4.4
30	58	2	3.4

*Two with urinary symptoms

and, of those, two presented within one day, one within two days, and one within three days of the most recent coitus. Fourteen patients were not asked the date of their last coitus.

Details of patients with UTI and the organisms isolated are shown in Table 3. In all but four instances the organism causing the infection was *Escherichia coli*; in the other instances the organisms were *Klebsiella* species (2), *Staphylococcus albus*, and a *Micrococcus* species. Sixteen of the 21 organisms were sensitive to ampicillin, sulphonamides, trimethoprim, and nitrofurantoin. Four of the organisms were resistant to sulphonamides. Only four of the 21 patients had urinary symptoms; most presented with vaginal discharge or because the sexual contact had symptoms.

Discussion

This study indicates that 4.9% of female patients attending a clinic for STD, mostly for the first time, had significant bacteriuria. This is a slightly higher percentage than would be expected in a population of healthy, young women. Asscher *et al.* (1969), for

example, found significant bacteriuria in 3% of 3578 healthy women aged 20-65. Grüneberg *et al.* (1969) found significant bacteriuria in 4.4% of 8907 women attending an antenatal clinic. In this study the prevalence of UTI almost equalled that of gonorrhoea (7%). This raises the question of the value of screening. There is no doubt that UTI can cause problems in pregnancy. Kincaid-Smith and Bullen (1965), for example, found that fetal loss and prematurity (fetal weight less than 2500 grams) were significantly increased in pregnant women with bacteriuria compared with women without. There is also a danger of pyelonephritis in pregnancy (Kass, 1960). There have been many reports that women with bacteriuria often have radiological evidence of renal abnormality. The investigation and treatment of women with bacteriuria detected by screening might help to reduce morbidity in a subsequent pregnancy. The women attending a STD clinic are, by virtue of their age and sexual activity, very likely to become pregnant; in fact, pregnancy is often first diagnosed at the clinic. All new patients have a routine urine examination for protein and sugar, and the screening of urine samples for the presence of significant bacteriuria is a quick and simple procedure (Leigh and Williams, 1964). Reliance on symptoms is insufficient; only four of the 21 women with positive cultures had urinary tract symptoms. However, treatment of UTI is often followed by reinfection, and some workers are sceptical of the value of screening for bacteriuria in women who are not pregnant (Asscher *et al.*, 1969).

Table 3 Symptoms, urinary pathogens, and other diagnoses in patients with UTI

Case no.	Symptoms	Species	Sensitivity to				Other diagnoses
			Ampicillin	Sulphonamides	Trimethoprim	Nitrofurantoin	
1	Vaginal discharge	<i>E. coli</i>	S	S	S	S	Trichomoniasis
2	Asymptomatic	<i>E. coli</i>	S	S	S	S	—
3	Dysuria and haematuria	<i>E. coli</i>	S	S	S	S	—
4	Recurrent soreness	<i>E. coli</i>	R	R	R	S	—
5	Vaginal discharge	<i>E. coli</i>	S	S	S	S	Trichomoniasis
6	Vaginal discharge	<i>E. coli</i>	S	S	S	S	—
7	Vaginal discharge	<i>Klebsiella</i> sp.	R	R	S	S	Candidosis
8	Genital warts	<i>E. coli</i>	S	S	S	S	—
9	Dysuria and frequency	<i>E. coli</i>	S	S	S	S	Candidosis
10	Dysuria and frequency	<i>E. coli</i>	S	S	S	S	Candidosis
11	Asymptomatic	<i>S. albus</i>	S	S	S	S	—
12	Asymptomatic	<i>E. coli</i>	S	R	S	S	Trichomoniasis
13	Asymptomatic	<i>E. coli</i>	S	S	S	S	—
14	Vaginal discharge	<i>E. coli</i>	S	S	S	S	—
15	Asymptomatic	<i>E. coli</i>	S	S	S	S	Candidosis
16	Vaginal discharge	<i>E. coli</i>	S	S	S	S	—
17	Irritation and frequency	<i>Micrococcus</i> sp.	R	S	S	S	—
18	Tertiary syphilis	<i>Klebsiella</i> sp.	M	R	S	S	Syphilis
19	Asymptomatic	<i>E. coli</i>	S	S	S	S	—
20	Asymptomatic	<i>E. coli</i>	S	S	S	S	—
21	Asymptomatic	<i>E. coli</i>	S	S	S	S	Candidosis

R=resistant, S=sensitive, M=? effective in high dose

In this study we have shown (Table 2) that women with UTI are more likely to have had coitus during the previous 24 hours than are women without UTI. The explanation for this could be either that sexual intercourse causes a short-lasting bacteriuria which is then detected on screening or that women with UTI have coitus more frequently than uninfected women. In either case, coitus seems in some way to be a precipitating factor in the development of most episodes of UTI occurring in young women. This suggestion is often made but is rarely supported by objective data.

We have been unable to demonstrate in this study any relationship between the number of sexual partners in the previous year and the chance of a woman having UTI. There was, however, a greater prevalence of gonorrhoea and trichomoniasis in the more promiscuous group of women. These findings suggest that UTI may be related more to the frequency of coitus than to the number of sexual partners and is therefore probably not a sexually transmitted disease.

We would like to thank the staff of the Departments of Genitourinary Medicine and Microbiology, University College Hospital, for their help in this work and to Dr J. D. Oriel for allowing us to study patients under his care.

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Early malignant syphilis observed during infection and reinfection in the same patient

K. LEJMAN AND Z. STARZYCKI

From the Dermatological Clinic, Medical Academy, Kraków, Poland

SUMMARY Malignant syphilitic infections occurring in the same woman at an interval of 38 months is reported. The first infection was diagnosed as secondary papulopustular and ecthymatous syphilis. The reinfection was more severe, taking the form of secondary ulcerative syphilis with fever, general deterioration, and loss of weight. Numerous *Treponema pallidum* were present in the serum expressed from the papulopustular lesions during the first infection, but they were absent from the secretion expressed from the margins of the ulcerative syphilides during the reinfection. Many treponemes were present in the plasma cell infiltrates in histopathological sections of the biopsy specimens taken from the margin of the ulcers.

Introduction

The destructive, ulcerating, and necrotic syphilides in early syphilis, commonly called malignant syphilis, are rare nowadays, although lately there have been reports of them (Agache *et al.*, 1970; Degos *et al.*, 1970; Laugier *et al.*, 1970; Lejman and Starzycki, 1972; Jarowskij and Cholin, 1973; Buck, 1974; Petrozzi *et al.*, 1974; Sehgal and Rege, 1974; Lomyskin and Lanne, 1975; Pariser, 1975).

We have, however, been unable to find any report concerning malignant syphilitic infection and reinfection in the same patient.

Case report

FIRST INFECTION

The patient, a 38-year-old woman, was admitted on 11 July 1969 with a generalised eruption present for at least two weeks. She complained of fever, headache, and photophobia of the left eye. She was a prostitute and admitted sexual contact with many partners, none of whose personal details was known to her.

Clinical examination

The patient was of normal build and moderately nourished. She was 1.66 m tall and weighed 52 kg. The axillary temperature was 38.2°C, and the pulse rate was 96 beats a minute.

The eruption covered the surface of her body (except for the palms of her hands and soles of her feet) particularly the trunk, abdomen, and genitals. The lesions ranged from papulopustular to papulonecrotic, and many were covered with dark, adherent crusts (Fig. 1). After desquamation of the necrotic crusts scattered, small, ecthymatous lesions could be seen. All the lesions had an erythematous base. On the genitals many of the pustules were denuded and oozing (Fig. 2). There were no lesions of the oral mucosa or of the anal region. She had iridocyclitis of the left eye.

Laboratory investigations

Darkground examination of the serum from the pustular lesions on the genitals and forearms showed typical, numerous, motile *T. pallidum*. Haemoglobin was 11.2 g/dl (11.2 g/100 ml); erythrocyte sedimentation rate was 88 mm in 1 hour; and white blood count (WBC) was $6.1 \times 10^9/l$ ($6.1 \times 10^3/mm^3$) with a normal differential count.

Serological tests

The Wassermann reaction (WR) was strongly positive; the Venereal Disease Research Laboratory (VDRL) test gave positive results at a titre 1:64; the *T. pallidum* immobilisation (TPI) test result was positive at 100% immobilisation; the result of the fluorescent treponemal antibody *200 (FTA-200) test was positive at a titre 1:24 000; the fluorescent treponemal antibody absorption (FTA-ABS) test also gave positive results.

Address for reprints: Professor K. Lejman, Klinika Dermatologiczna AM, ul. Kopernika 17, 31-501 Krakow, Poland

Received for publication 14 November 1977



Fig. 1 Early secondary papulopustular and ecthymatous syphilides in the first infection.

Lumbar puncture

The cerebrospinal fluid (CSF) cell count was 3 cells/mm³ and the protein was 0.12 g/l (12 mg/100 ml); the Pándy, Nonne-Apel, and Weichbrodt tests gave negative results as did the WR, VDRL, and TPI tests; the FTA test was positive at a titre 1:50.

Diagnosis

- Secondary malignant papulopustular and ecthymatous syphilis was diagnosed.

Treatment and course of the illness

The patient was given 600 000 units procaine penicillin daily to a total of 21 megaunits. Seven hours after the first injection the body temperature



Fig. 2 Papulopustular syphilides on the genitals in the first infection.

rose to 40.8°C, returning to normal after 20 hours. The eruption healed slowly. The ophthalmological features disappeared completely.

On discharge from the clinic on 14 August the serological tests showed no change. The patient then moved her address and did not attend for follow-up examination. Fifteen months later, however, she attended the venereological dispensary, and the result of her VDRL test was negative.

REINFECTION

On 21 October 1972, 38 months after discharge from the clinic, the patient was again admitted with severe, ulcerating, partly necrotising, scattered cutaneous lesions.

She had noticed the onset of the lesions two months earlier, and since then had lost 8 kg in weight. The rash had been preceded by prodromal symptoms lasting one month—such as fever up to 38–39°C, severe headaches, loss of appetite, and general weakness.

Clinical examination

She was undernourished; her weight was 48.5 kg, and the axillary temperature was 37.8°C.

The lesions (11 in number) were spread on the extremities (Fig. 3), the right shoulder (Fig. 4), the neck, the interscapular region, and the right hip. The lesions were ulcerating and had polycyclic margins encircled with inflammatory borders and covered with necrotic crusts. Some of them, particularly those on the right shoulder, were composed of confluent ulcers resembling noduloulcerative tertiary syphilides. The lesions were slightly tender and secreted sticky, foul-smelling, colourless exudate.

Laboratory investigations

Repeated darkground examination of the exudate from the ulcers, as well as of the tissue fluid aspirated from the base, were negative for *T. pallidum* and sterile for fungi and *Candida albicans*. *Staphylococcus aureus* coagulase-positive, β haemolytic *Streptococcus pyogenes*, and *Corynebacterium pseudodiphtheriae* were cultured from the ulcers.

Haemoglobin was 12.1 g/dl (12.1 g/100 ml); WBC was $8.4 \times 10^9/l$ ($8.4 \times 10^3/mm^3$) with normal differential count; and erythrocyte sedimentation rate was 97 mm in one hour.

Serological tests

The WR was strongly positive; the VDRL test gave positive results at a titre 1:64; the TPI test

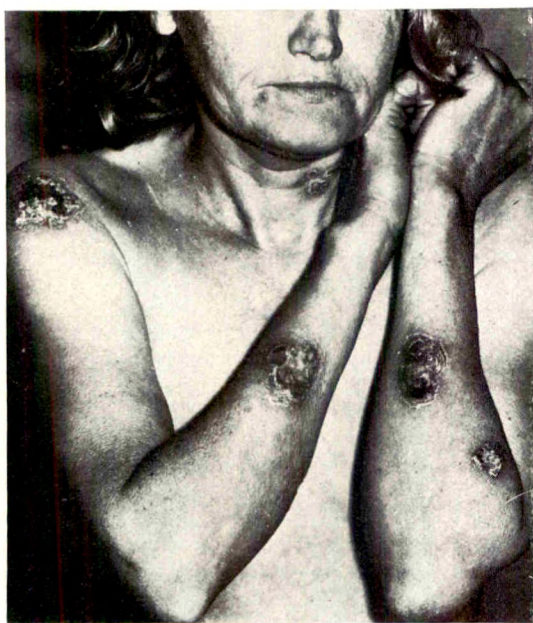


Fig. 3 Ulceronecrotic syphilides on the extremities in the reinfection.



Fig. 4 Ulceronecrotic lesion similar to the tertiary syphilide on the right shoulder in the reinfection.

gave positive results with 100% immobilisation; and the FTA-₂₀₀ test gave positive results at a titre 1:4000.

Lumbar puncture

The CSF cell count was 5 cells/mm³; the protein was 0.26 g/l (26 mg/100 ml); the Pándy, Nonne-Apelt, and Weichbrodt tests gave results that were weakly positive. The WR and VDRL results were negative; the TPI result was positive with 100% immobilisation; and the FTA-₂₀₀ result was positive at a titre 1:50.

Histopathology

Two biopsies from the margins of the lesions on the trunk and the hip were taken. Sections from both specimens were stained with haematoxylin and eosin and with modification of the Krajian silver impregnation method (Walter *et al.*, 1969).

Sections stained with haematoxylin and eosin showed that the epidermis was hyperkeratotic and

partly parakeratotic, and contained scattered intra-corneal pustules. The malpighian rete was irregularly acanthotic, and the outline between epidermis and dermis was in places hazy. The capillary vessels of the papillary layer of the skin were greatly dilated and filled with blood. In the underlying layers of the skin the blood vessels were in places disrupted causing haemorrhages, and some of these were blocked by thrombi. The collagenous structure of the skin was almost completely distorted by a dense plasma cell infiltrate with a few lymphocytes and histiocytes (Fig. 5).

In the sections stained with modified Krajan's method masses of treponemes were present within the infiltrates of plasma cells (Fig. 6) as well as in the walls of the blood vessels and, in a few places, within the intravascular thrombi. The epidermis was free from treponemes.

Diagnosis

Reinfection in the form of early malignant, ulceronecrotic syphilis was diagnosed.

Experimental investigations

A healthy, seronegative (including WR, VDRL, TPI, FTA-₂₀₀, and FTA-ABS tests) rabbit was

inoculated intratesticularly with 0.3 ml of exudate and aspirate from the ulcerated lesions. Ten days later the animal remained asymptomatic; the VDRL test gave negative results but the WR became positive. One month after the inoculation the results of all the serological tests mentioned above, except the TPI test, became positive (the VDRL test at a titre 1:4 and the FTA-₂₀₀ test at a titre 1:300). The rabbit died two months after inoculation without clinical signs.

Treatment and course of the illness

The patient was given 600 000 units procaine penicillin daily for three days, then 900 000 units daily up to a total dose of 30 megaunits. The first injection of penicillin was followed by a rise in temperature to 40.1°C, with concomitant chills and headaches. A few days later the foul-smelling exudate diminished, but healing was slow.

Observation after treatment

Two months after the patient's discharge the result of the VDRL test was positive at a titre 1:32 and that of the FTA-₂₀₀ test positive at a titre 1:16 000. The patient was not seen again until 21 October 1975. The VDRL result was still strongly positive; the titre of the FTA-₂₀₀ test, however, had increased to 1:64 000.

From April to December 1976 the woman remained under observation, but there was no clinical evidence of recurrence. The CSF cell count was normal; the VDRL and FTA results in the CSF were negative; but the serum VDRL still gave positive results at a titre 1:64; and the FTA-₂₀₀ test result was positive at a titre 1:1300. In January 1977 she began further treatment with procaine penicillin in a municipal venereological dispensary.

Discussion

Treatment of the first infection with penicillin was followed by a negative VDRL result one year after the end of the treatment. During the next 26 months the patient had had ample opportunity to acquire a fresh infection, and it seems reasonable to presume the second episode was a reinfection. The course of the reinfection was much more severe, and the signs of malignancy were accentuated by the appearance of ulcerated and even necrotic lesions, resembling the tertiary syphilides; furthermore the general condition of the patient was worse.

The negative results in the search for *T. pallidum* in the exudates and in the aspirate of the ulcerous lesions during reinfection contrasts with the enormous masses of these organisms in the plasma cell

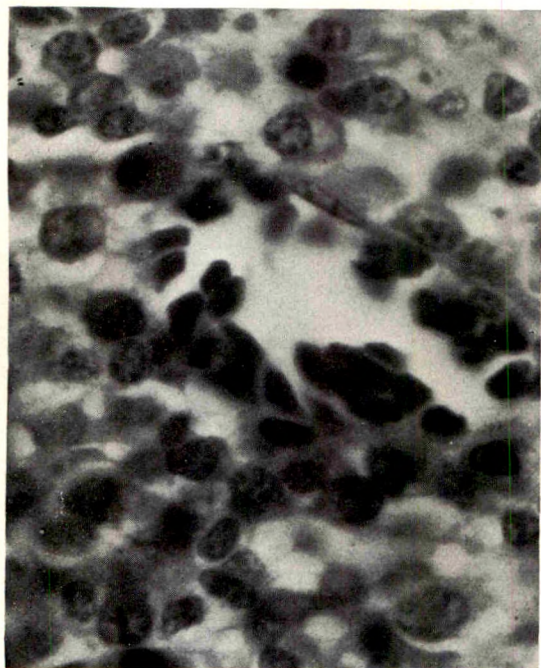


Fig. 5 Dilated capillary vessel in the papillary layer filled partially with erythrocytes and surrounded almost exclusively by plasma cells (section stained with haematoxylin and eosin; $\times 1500$ magnification).

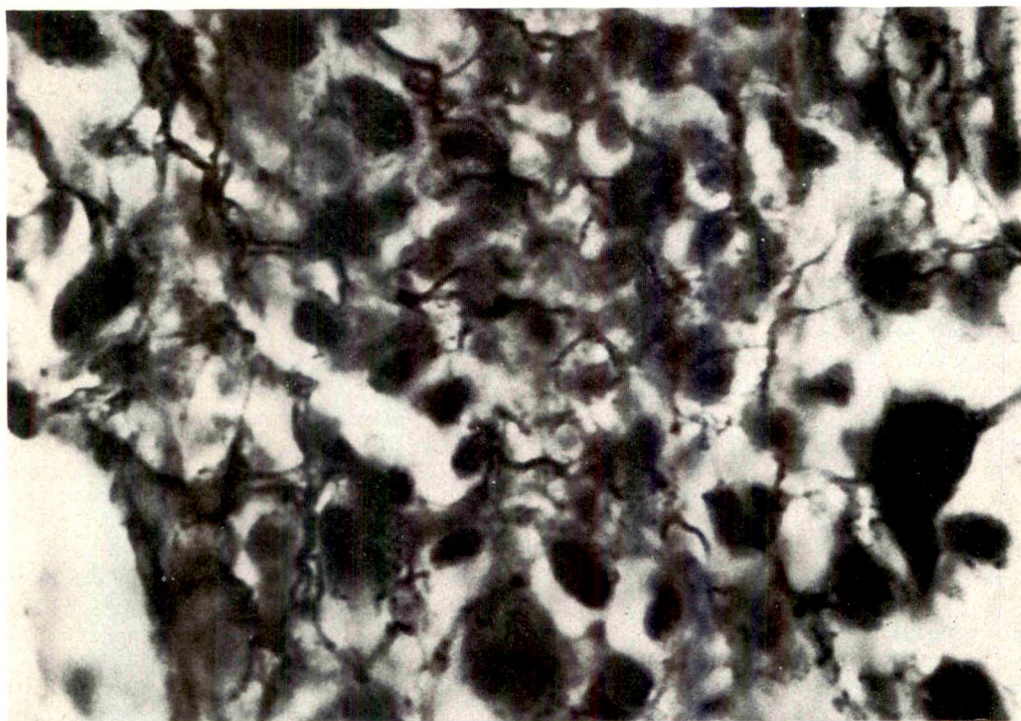


Fig. 6 Enormous masses of *T. pallidum* within the infiltrate of plasma cells from the margin of an ulcerous lesion (section stained with modified Krajan silver impregnation method; $\times 1500$ magnification).

infiltrates seen in the histopathological section stained with the modified silver impregnation method from the destructive lesions. The absence of the treponemes in the expressed serum may be explained by their deeper localisation.

Although some of the lesions during reinfection resembled tertiary lesions, their histopathological and microbiological features corresponded to the early stage of the disease.

The lack of clinical response in the infected rabbit suggests that the treponemal isolates were not equally virulent. This in turn suggests that the malignant course of the patient's disease reflects the body's response against treponemal infection.

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Correspondence

Epidermal antibodies in secondary syphilis

TO THE EDITOR, *British Journal of Venereal Diseases*

Sir,

Syphilitic patients may show symptoms of an 'aberrant' antibody synthesis. Antibodies bearing evidence of this are rheumatoid factor and antibodies against erythrocytes. Wright *et al.* (1970) have found that sera of patients with early syphilis contain antibodies, which stain cell mitochondria in a fluorescent test. These antibodies are not organ-specific and are characterised by their absorption with Venereal Disease Research Laboratory antigen, from which cardiolipin was shown to be the active ingredient.

When looking for autoantibodies in sera of patients with early syphilis (less than two years), we found fluorescence with several tissue substrates which could also be absorbed with cardiolipin.

The sera from 26 patients with untreated syphilis were tested by the indirect immunofluorescence technique (IFT) for the presence of autoantibodies. Six of these 26 patients had seronegative primary syphilis, six serolabile primary syphilis, two seropositive primary syphilis, seven secondary syphilis, and five early latent syphilis. The serological subdivision of primary syphilis was made by applying antilipoidal and antitreponemal tests (Kolmer, VDRL, Reiter protein complement-fixation, and *Treponema pallidum* immobilisation): seronegative primary syphilis was diagnosed when all results were negative; serolabile primary syphilis when some were positive; and seropositive primary syphilis when all were positive. The tissue substrates used for the IFT for autoantibody determination were unfixed, frozen, human parotid, adrenal, and toxic thyroid glands and rat heart, stomach, striated muscle, and kidney. Guinea-pig lip was used as substrate for skin-antibodies. We found that:

1. The sera from all the patients with seropositive primary or secondary syphilis produced a strong granular fluorescence of

guinea-pig lip. This fluorescence differed from the pattern regularly found with the sera from patients with pemphigus or parapemphigus.

2. The same sera also produced a strong smooth muscle fluorescence, which was different from the pattern that is observed with sera in active chronic hepatitis or primary biliary cirrhosis.

The sera from patients with seropositive primary or secondary syphilis also caused weak fluorescence with striated muscle and heart muscle and with parotid and thyroid glands.

The fluorescence in epidermis, smooth muscle, striated muscle, heart muscle, parotid and thyroid glands could be abolished by preabsorption of the sera with cardiolipin. Control absorption with lecithin and phosphate buffered saline was negative. Treatment of the patients with penicillin led to a decrease in the titre of the antibodies.

In the sera from patients with seronegative or serolabile primary syphilis and in the sera from three of five patients with early latent syphilis no such antibodies could be found with any of the substrates tested, although occasionally doubtful fluorescence of smooth muscle, epidermis, striated muscle, heart muscle, and thyroid gland was seen. In the two other cases of early latent syphilis the sera showed fluorescence of smooth muscle and epidermis, whereas fluorescence with other substrates was weak or absent.

Unlike Wright *et al.* (1970) we did not find a pattern of mitochondrial fluorescence in the distal tubules of kidney. This may be caused by the fact that we used rat kidney instead of human kidney. Although with rat kidney mitochondrial fluorescence in sera from patients with syphilis has been described (André *et al.*, 1973). As nearly all types of fluorescence could be absorbed with cardiolipin we found with the indirect immunofluorescence technique no indications that an infection with *Treponema pallidum* induces the synthesis of antibodies against several autoantigens as suggested by

Allison (1973) and recently by Primi *et al.* (1977). These findings confirm those of Doniach (1976).

Yours faithfully,

N. H. L. de Jong*, J. J. van der Sluis, J. van Dijk†, and T. E. W. Felkamp†

*Department of Dermatology, Erasmus University, Rotterdam, The Netherlands

†Central Laboratory of the Red Cross Bloodtransfusion Service, Department of Auto-Immune Diseases, Amsterdam, The Netherlands

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Tetracycline treatment for non-specific urethritis

TO THE EDITOR, *British Journal of Venereal Diseases*.

Sir,

Dr Fowler (1978) disagrees with my advocacy of a long follow-up for the assessment of treatment of non-specific urethritis, stating that it 'was shown to be unnecessary in a study (Fowler, 1970) in which the follow-up was not the one year Mr Simopoulos finds so admirable, but three years, and the long-term findings proved of no help in assessing treatment'. In saying this, Fowler is doing scant justice to his own paper. He then refers to a time 'in the past when the aetiology

and the natural course of NSU were obscure and we needed treatment for the condition as a whole'. Humility bids us accept this as being as good a description of the present as it is of the past.

Masterton and Schofield (1978) take me to task for a misstatement about their arithmetic, and I am happy to acknowledge my mistake and to withdraw the criticism I associated with it. It was the only adverse criticism of their paper, which has been unjustly neglected.

Taylor-Robinson *et al.* (1978) agree with me that there are serious flaws 'in studies which have been carried out to determine the effectiveness of tetracyclines'; and that was my main point. They go on, however, to chide me for 'a remarkable lack of appreciation of the

problems'. Firstly, they point out that 'a placebo-controlled study would now raise severe ethical problems'. This is arguable, but if one succumbs to the argument, a non-antibiotic drug could be used, as I suggested on p. 230 of my paper (Simopoulos, 1977). Secondly, they state that 'the sexual activity of patients cannot be accurately monitored over a long period unless there are most unusual circumstances (for example, aircraft carriers, Antarctica)'. Far from not appreciating this problem, I refer to it in my paper.

Yours faithfully,

J. Ch. Simopoulos

St. Catherine's College, Oxford

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Abstracts

These selected abstracts and titles from the world literature are arranged in the following sections:

Syphilis and other treponematoses
(Clinical and therapy; serology and biological false positive phenomenon; pathology and experimental)
Gonorrhoea
(Clinical; microbiology; therapy)

Non-specific genital infection
Reiter's disease
Trichomoniasis
Candidosis
Genital herpes
Other sexually transmitted diseases

Public health and social aspects
Miscellaneous

Syphilis and other treponematoses (Clinical and therapy)

Secondary syphilis revealed by rheumatic complaints

J. C. GERSTER, A. WEINTRAUB, T. L. VISCHER, AND G. H. FALLET (1977). *Journal of Rheumatology*, 4, 197-200

Six patients (five men and one woman) are described, who consulted rheumatologists with varied rheumatic complaints. Four patients had subacute synovitis with effusion, frequently associated with vague arthralgias, and five had back pain which was more severe at rest. Most of the patients had some clinical signs of secondary syphilis, such as roseola, loss of hair, or lymphadenopathy, and the results of their serological tests for syphilis were strongly positive. As no other cause for these rheumatic complaints could be found, secondary syphilis was considered responsible for them. In all cases the rheumatic complaints cleared with specific treatment.

Authors' summary

Syphilis in Australian aborigines in the Northern Territory

D. S. JACOBS (1978). *Medical Journal of Australia*, 1, 10-11

Syphilis (Pathology and experimental)

Anabolic potential of virulent *Treponema pallidum*

J. B. BASEMAN AND N. S. HAYES (1977). *Infection and Immunity*, 18, 857-865

Selective *in vitro* response to thymus-deprived lymphocytes from *Treponema pallidum*-infected rabbits

C. S. PAVIA, J. D. FOLDS, AND J. B. BASEMAN (1977). *Infection and Immunity*, 18, 603-611

Gonorrhoea (Clinical)

Gonorrhoea in women: Diagnostic, clinical and laboratory aspects

D. BARLOW AND I. PHILLIPS (1978). *Lancet*, 1, 761-764

Examination of the case notes of all women seen at a large metropolitan clinic during 1976 showed 607 episodes of gonorrhoea (92.3% of all such cases seen in the hospital), of which three were in prepubertal girls. Gonorrhoea occurred more often and at an earlier age in Negroids than in Caucasians. In about 30% of patients gonococci could be found in only one of the sites tested (cervix 18%, urethra 6%, rectum 4.8%, and throat 1.5%). Microscopical examination of Gram-stained cervical and rectal samples was of value, but that of urethral samples made no significant contribution to the diagnosis. Of the gonococcal isolates 31% showed diminished sensitivity to penicillin, but none showed significant resistance to spectinomycin, kanamycin, or sulphamethoxazole. The complication rate was lower than that reported from the United States. Overall, 40% of patients were symptom-free. The presence of other infection significantly increased the probability of a patient with gonorrhoea having symptoms. 'Epidemiological' treatment would have led to the unnecessary treatment of 142 female patients and would have included only four of 16 patients with gonorrhoea

who defaulted before treatment could be given.

Authors' summary

Gonococcal perihepatitis in a female adolescent

L. G. MCLAIN, M. DECKER, D. NYE, S. MEHTA, AND R. LONDON (1978). *Journal of the American Medical Association*, 239, 339

Gonococcal proctitis in a married woman

N. J. FLUMARA (1977). *Journal of the American Medical Association*, 238, 2718

The incidence of gonorrhoea in urban Rhodesian black women

R. WEISSENBERGER, A. ROBERTSON, S. HOLLAND, AND W. HALL (1977). *South African Medical Journal*, 52, 1119-1120

Containing gonorrhoea epidemic

P. O. ROBERTS (1978). *American Journal of Public Health*, 68, 1

Gonorrhoea (Microbiology)

Simple method for distinguishing gonococcal colony types

E. JUNI AND G. A. HEYM (1977). *Journal of Clinical Microbiology*, 6, 511-527

A new procedure for the identification of gonococcal colony types is described which employs a dissecting microscope with a fluorescent lamp and concave mirror, critical adjustment of which produces a darkfield effect. This could be of use to anyone who wants to distinguish gonococcal colony types.

Brian Evar

Growth on Congo red agar: possible means of identifying penicillin-resistant non-penicillinase-producing gonococci

S. M. PAYNE AND R. A. FINKELSTEIN (1977). *Journal of Clinical Microbiology*, 6, 534-535.

A standard growth medium (GC medium base plus 1% defined supplement) containing 0.01% Congo red dye was found to support the growth of gonococci only if the penicillin minimal inhibitory concentration (MIC) was 0.5 µg/ml or greater. Penicillinase-producing organisms and organisms more sensitive to penicillin, did not grow. In an earlier paper, the authors had reported that meningococci formed red colonies on an agar medium containing Congo red, whereas gonococci failed to grow. These observations are of potential value in the rapid identification of penicillin-resistant gonococci, unless they produce penicillinase.

Brian Evans

Trends and seasonality of antibiotic resistance of *Neisseria gonorrhoeae*

H. W. JAFFE, A. A. ZAIDI, C. THORNBERRY, G. H. REYNOLDS, AND P. J. WEISNER (1977). *Journal of Infectious Diseases*, 136, 684-688

An overall increase in sensitivity to penicillin G, ampicillin, tetracycline, and spectinomycin is reported for pretreatment gonococcal isolates in the United States between 1972 and 1975. This reverses the trend towards greater resistance to penicillin and tetracycline recorded between 1955 and 1970. Furthermore, resistance to penicillin and tetracycline proved highest in the winter months when the incidence of gonorrhoea was lowest, which the authors suggest may be due to increased general usage of antibiotics removing the more sensitive strains fortuitously.

Brian Evans

Studies on gonococcus infection. XI. Comparison of *in vivo* and *in vitro* association of *Neisseria gonorrhoeae* with human neutrophils

G. KING, J. F. JAMES, AND J. SWANSON (1978). *Journal of Infectious Diseases*, 137, 38-43

Studies on gonococcus infection. XII. Color colony and opacity variants of gonococci.

J. SWANSON (1978). *Infection and Immunity*, 19, 320-331

Studies on gonococcus infection. XIII. Occurrence of color/opacity colonial variants in clinical cultures

J. F. JAMES AND J. SWANSON (1978). *Infection and Immunity*, 19, 332-340

Acrylamide gel electrophoresis of proteins of *Neisseria gonorrhoeae* as an epidemiological tool

R. C. NOBLE AND S. C. SCHELL (1978). *Infection and Immunity*, 19, 178-230

Antigonooccal IgA in gonorrhoea

A. A. GLYN AND C. ISON (1978). *Lancet*, 1, 557

Cell envelope of *Neisseria gonorrhoeae*: penicillin enhancement of peptidoglycan hydrolysis

W. S. WEGENER, B. H. HEBELER, AND S. A. MORSE (1977). *Infection and Immunity*, 18, 717-846

Sensitivity of gonococci to penicillin G in the Canton of Berne, 1972-1977

A. NOVAK (1978). *Schweizerische medizinische Wochenschrift*, 108, 98-100

Nonspecific genital infection

Chlamydia trachomatis infection and venereal disease

G. JOHANNISSON, B. EDMAR, AND E. LYCKE (1977). *Acta dermatovenereologica*, 57, 455-458

Chlamydia trachomatis was isolated by the irradiated McCoy cell technique from 44 out of 103 men with nongonococcal urethritis and from 11 out of 15 patients with postgonococcal urethritis. In women attending the venereal diseases clinics, chlamydial infection was observed in 49 (38%) out of 130 patients, an infection incidence of the same order of magnitude as the one noted for gonococcal infection (40%). In 19% both infections occurred simultaneously. Treatment with tetracycline eliminated symptoms and chlamydial infection in almost all cases. The significance of the findings is discussed.

Authors' summary

Chlamydia trachomatis as a cause of acute idiopathic epididymitis

R. E. BERGER, E. R. ALEXANDER, G. D. MONDA, J. ANSELL, G. MCCORMICK, AND K. K. HOLMES (1978). *New England Journal of Medicine*, 298, 301-304

To assess the aetiological role of *Chlamydia trachomatis* and other micro-organisms in 'idiopathic' epididymitis, 23 men

underwent microbiological studies, including cultures of epididymal aspirates in 16. Eleven of 13 men under the age of 35 years had *C. trachomatis* infection whereas eight of 10 over 35 had coliform urinary tract infection. Cultures of epididymal aspirates yielded *C. trachomatis* alone in five of six men under 35, and coliform bacteria alone in five of 10 over 35. These results suggest that *C. trachomatis* is the major cause of 'idiopathic' epididymitis, and coliform bacteria the major cause of epididymitis in older men. Expressible urethral discharge and inguinal pain were more common in the chlamydial cases, whereas concurrent genitourinary abnormality and scrotal oedema and erythema occurred more commonly in the coliform cases. The morbidity attributable to *C. trachomatis* is as serious as that attributable to *Neisseria gonorrhoeae*.

Authors' summary

Chlamydial pharyngitis?

W. R. BOWIE, E. RUSSELL ALEXANDER, AND KING K. HOLMES (1978). *Journal of the American Venereal Disease Association*, 4, 140-141

Among 118 women who were sexual contacts of men with nongonococcal urethritis, the practice of fellatio correlated with symptoms of a sore throat. Oropharyngeal cultures for *Chlamydia trachomatis* were negative in all women, including 11 women who practised fellatio and whose partners were known to have nongonococcal urethritis due to *C. trachomatis*. The study does not support a major role for *C. trachomatis* as a cause of sore throat in women who practise fellatio.

Authors' summary

Chlamydia trachomatis infant pneumonitis. Comparison with matched controls and other infant pneumonitis

H. R. HARRISON, M. G. ENGLISH, C. K. LEE, AND E. R. ALEXANDER (1978). *New England Journal of Medicine*, 298, 702-708

We determined the prevalence of *Chlamydia trachomatis* infection in 30 consecutive hospitalised infants less than six months of age with pneumonitis and in 28 matched controls (nine of 30 compared with one of 28, $P < 0.05$).

In comparing 16 cases of pneumonitis due to *C. trachomatis* with 27 not due to

that agent, we found several distinguishing clinical and laboratory features: *C. trachomatis* was highly correlated with radiographic hyperinflation, prolonged cough, and congestion; more than 400 eosinophils/mm³, and serum IgG greater than 500 mg/dl and IgM greater than 110 mg/dl. *C. trachomatis* was responsible for 13 of 21 cases seen at 3–11 weeks compared with three of 22 seen at other ages. Antibody to *C. trachomatis* in tears (13 of 14 compared with two of 27), nasopharynx (12 of 14 compared with one of 27) and blood (16 of 16 compared with two of 23) was specific for *C. trachomatis* pneumonitis.

C. trachomatis is prevalent among hospitalised infants with pneumonitis. Conjunctival infection precedes *C. trachomatis* pneumonitis more commonly than has previously been thought.

Authors' summary

Isolation of chlamydiae in untreated and cytochalasin B treated McCoy cells

J. J. O'NEILL, B. M. MCLEAN, AND M. H. HAMBLING (1978). *Journal of Clinical Pathology*, 31, 183–184

A comparison was made between untreated McCoy cells and McCoy cells treated with cytochalasin B for the isolation of chlamydiae of subgroup A. Chlamydiae were isolated in both cell systems from 125 specimens, whereas six agents were isolated only in untreated cultures, and seven agents were isolated only in cytochalasin B treated cultures.

Authors' summary

Antichlamydial antibody in genital exudates of men and women with non-gonococcal genital infections

K. M. NG, D. M. GRAHAM, J. R. L. FORSYTH, AND K. F. BRENNAN (1978). *Lancet*, 1, 507

The role of antibody estimation in chlamydial infections is controversial. This letter reports the findings of a study to compare chlamydial isolation with single estimations of antichlamydial antibodies in the discharges of men and women with genital infections. In the men, duplicate alginate urethral swabs were taken. One was cultured on HeLa 229 cell monolayers for chlamydial isolation, and the other placed in phosphate buffer; after shaking with glass beads, the supernatant was titred for immunofluorescent antibody against standard serotypes of *C. trachomatis*.

C. trachomatis was isolated from 27% of the male population studied. In 64% of these, specific antibody, matching the serotype of the isolate, was detected (the titres are not stated). Antibodies to genital isolates were also detected in 17% of *C. trachomatis* negative men, with some overlap to more than one serotype of *C. trachomatis*. Of five women investigated, three yielded antichlamydial antibody, but no isolates were obtained after attempted culture. Clearly further study is required to establish the place of this work in the routine diagnosis of chlamydial genital infection. Results obtained on a single estimation of antibody must be interpreted with caution. Antibody detected may not reflect current infection in the absence of a specific isolate of *C. trachomatis*. The absence of antibody may merely reflect the acute nature of the infection.

G. L. Ridgway

Antibiotic susceptibility of *Chlamydia trachomatis*

H. J. BLACKMAN, C. YONEDA, C. R. DAWSON, AND J. SCHACHTER (1977). *Antimicrobial Agents and Chemotherapy*, 12, 673–677

The antibiotic susceptibility of *Chlamydia trachomatis* isolates was determined in a tissue culture system. Representatives of all currently recognised serotypes of trachoma-inclusion conjunctivitis agents were tested. Tetracycline and erythromycin yielded similar results, with 1.0 µg/ml preventing chlamydial replication. Rifampin was the most active antibiotic, with 0.25 µg/ml completely suppressing inclusion formation of all strains. Fifty per cent end points were usually achieved at one-fourth to one-eighth of the suppression level. Penicillin was not as effective, and the assays were often irregular. Antibiotic susceptibility of these chlamydiae was essentially the same, regardless of serotype, anatomical site infected, geographic origin, or antibiotic use in the community.

Authors' summary

The incidence of tetracycline-resistant strains of *ureaplasma urealyticum*

R. T. EVANS AND D. TAYLOR-ROBINSON (1978). *Journal of Antimicrobial Chemotherapy*, 4, 57–63

Ureaplasma strains isolated from the urethras of men with non-gonococcal

urethritis were investigated for decreased sensitivity to oxytetracycline and minocycline. Of the 141 strains isolated, 14 (9.9%) were deemed resistant. The strains were found to belong to most of the known serotypes. Resistance was defined as a minimal inhibitory concentration of equal to or greater than 0.6 µg/ml to oxytetracycline, and equal to or greater than 0.16 µg/ml to minocycline. Attempts were made to induce resistance to minocycline in four sensitive strains by a multiple passage technique. While decrease in sensitivity was demonstrable, it did not attain the degree of resistance found in the naturally occurring strains. During these experiments the organism remained fully sensitive to erythromycin. Attempts to transfer natural resistance to sensitive strains were not successful.

In their general discussion the authors comment that in the metabolism-inhibition test, which is widely used to detect serum antibody, tetracycline in serum may inhibit the test organism. They recommend that naturally occurring resistant strains should be used in this test, as many patients will be receiving tetracyclines when serum samples are collected.

G. L. Ridgway

Chlamydia infections (first of three parts)

J. SCHACHTER (1978). *New England Journal of Medicine*, 298, 428–435

Chlamydial infections (second of three parts)

J. SCHACHTER (1978). *New England Journal of Medicine*, 298, 490–495

Chlamydial infections (third of three parts)

J. SCHACHTER (1978). *New England Journal of Medicine*, 298, 540–549

Infections due to *Chlamydia*

J. R. HIEBER (1977). *Journal of pediatrics*, 91, 864–869

Reiter's disease

Role of *Chlamydia trachomatis* and HLA-B27 in sexually acquired reactive arthritis

A. C. KEAT, R. N. MAINI, G. C. NKWAZI, G. D. PEGRUM, G. L. RIDGWAY, AND J. T. SCOTT (1978). *British Medical Journal*, 1, 605–608

Inflammatory arthritis, tendinitis, and fasciitis after non-specific urethritis,

'sexually acquired reactive arthritis' (SARA), was studied prospectively in 531 men with nonspecific urethritis, with particular reference to the frequency of isolation of *Chlamydia trachomatis* and the presence of HLA-B27. Satisfactory cultures were obtained from the urethral swabs from 384 patients; HLA typing was performed on 482, of whom 30 (6%) were HLA-B27-positive. Arthritis developed in 16 patients, and five (36%) of the 14 with satisfactory cultures were positive for *C. trachomatis*; 135 of the patients without arthritis were also positive for *C. trachomatis*, an identical proportion. Seven (40%) of the 15 patients with arthritis who were HLA-typed were HLA-B27-positive.

Six of the 30 patients with HLA-B27 developed peripheral arthritis in contrast to only nine of the 452 patients lacking the antigen, suggesting a tenfold increased susceptibility. *C. trachomatis*, however, was no more prevalent in cultures from HLA-B27-positive men than from the others. Thus carriage of *C. trachomatis* is unlikely to be influenced by HLA-B27.

C. trachomatis may be an important pathogen in some cases of SARA but does not appear to be an exclusive trigger factor for this condition.

Authors' summary

Trichomoniasis

Interference of human spermatozoal motility by *Trichomonas vaginalis*

J. P. TUTTLE, JR, T. W. HOLBROOK, AND F. C. DERRICK (1977). *Journal of Urology*, **118**, 1024-1025

Candidosis

Treatment of vulvovaginal candidiasis in pregnancy: a comparative study

D. MCNELLIS, M. MCLEOD, J. LAWSON, AND S. A. PASQUALE (1977). *Obstetrics and Gynecology*, **50**, 674-678

Genital herpes

Retention of urine in anogenital herpetic infection

J. K. OATES AND P. R. D. H. GREENHOUSE (1978). *Lancet*, **1**, 691-692

In 17 patients, 15 women and two men, acute retention of urine developed in

association with an attack of anogenital herpes. Constipation, blunting of sensation over the second and third sacral dermatomes, and neuralgic pains in the same area (with absence of the bulbocavernosus reflex in some individuals) suggested localised lumbosacral meningo-myelitis with involvement of mainly sacral nerve roots. The urinary dysfunction persisted on average for 10 days, and in four patients was severe enough to warrant catheterisation. Anogenital herpes should always be considered as a possible cause of acute retention of urine in sexually active young people, and the possibility of occult herpetic infection of the cervix and rectum should be investigated.

Authors' summary

Herpes simplex virus type-1 and type-2 in ocular disease

D. NEUMANNHAFFELIN, R. SUNDMACHER, G. WOCHNIK, AND B. BABLOK (1900). *Archives of Ophthalmology*, **96**, 64-69

Treatment of genital infections with *Herpesvirus hominis*

H. G. ADAMS (1977). *Journal of the American Venereal Disease Association*, **4**, 160-162

Other sexually transmitted diseases

Genital herpes and cervical carcinoma (Editorial) (1978). *British Medical Journal*, **1**, 807

Genital warts

J. D. ORIEL (1977). *Journal of the American Venereal Disease Association*, **4**, 153-159

Intraurethral condylomata acuminata: management and review of literature

T. J. DEBENEDICTIS, J. L. MARMAR, AND D. E. PRAISS (1977). *Journal of Urology*, **118**, 767-769

Anorectal condylomata acuminata: a missed part of the condyloma spectrum

O. L. A. SCHLAPPNER AND E. A. SHAFFER (1978). *Canadian Medical Association Journal*, **118**, 172

Acute pelvic inflammatory disease: characteristics of patients with gonococcal and nongonococcal infection and evaluation of their response to

treatment with aqueous procaine penicillin G and spectinomycin hydrochloride

W. M. MCCORMACK, K. NOWROOZI, S. ALPERT, S. G. SACKEL, Y-H. LEE, E. W. LOWE, AND J. S. RANKIN (1978). *Journal of the American Venereal Disease Association*, **4**, 125-131

We studied 41 women with acute gonococcal pelvic inflammatory disease (PID) and 42 women with acute nongonococcal PID. Women with gonococcal PID were more likely to have become ill during the first 10 days of their menstrual cycle ($P < 0.05$), presented themselves for treatment sooner ($P < 0.05$), and were more severely ill than patients with non-gonococcal PID ($P < 0.05$). Patients were treated with aqueous procaine penicillin G or with spectinomycin hydrochloride for five days. Most of the patients with gonococcal disease responded to treatment. Neither drug, in the dosage used in this study, was highly effective in the treatment of acute nongonococcal PID. In all, 10 of 21 women with nongonococcal PID and only one of 19 women with gonococcal PID required retreatment for PID within 28 days ($P < 0.05$). Re-examination at an average of 17 months after treatment showed that women who had been treated for non-gonococcal PID were more likely to develop recurrent PID if the episode of PID treated in the study was not their first. Women treated for nongonococcal PID were also less likely to become pregnant ($P < 0.05$).

These data, which show that gonococcal and nongonococcal PID differ in initial clinical severity, response to treatment and long-term complications, support the concept that gonococcal PID and nongonococcal PID are separate clinical entities.

Authors' summary

Vaginal colonisation with *Corynebacterium vaginale* (*Haemophilus vaginalis*)

W. M. MCCORMACK, C. H. HAYES, B. ROSNER, J. E. EVARD, V. N. CROCKETT, S. ALPERT, AND S. H. ZINNER (1977). *Journal of Infectious Diseases*, **136**, 740-745

Vaginal cultures for *Corynebacterium vaginale* and confidential questionnaire were obtained from unselected young women who consulted a gynaecologist in a student health service. In all, 466 women

were studied, 150 (32.2%) of whom were colonised with *C. vaginale*. Logit analysis defined four factors that were significantly associated with colonisation with *C. vaginale*: non-white race, use of oral contraceptives, no history of marriage, and a history of pregnancy. Sexual experience had little influence on colonisation; *C. vaginale* was isolated from 16 (29%) of 56 sexually inexperienced women and from 40 (41%) of 98 women who had had sexual intercourse with six or more men. After a few patients with trichomoniasis were excluded, there was no association between colonisation with *C. vaginale* and an abnormal vaginal discharge, either as reported by the participant or as noted by the examining physician.

Authors' summary

Hepatitis B surface antigen (HBsAg) and antibody to HBsAg. Prevalence in homosexual and heterosexual men

D. E. DIETZMAN, J. P. HARNISCH, C. G. RAY, E. R. ALEXANDER, AND K. K. HOLMES (1977). *Journal of the American Medical Association*, **238**, 2625-2626

The prevalence rates of serum hepatitis B surface antigen (HBsAg) and antibody to HBsAg (anti-HB) were 5.6% and 34%, respectively, in 144 homosexual men in Seattle. Prevalence rates were only 0.9% and 3.6%, respectively, in 111 heterosexual male venereal disease clinic patients with nongonococcal urethritis, and also 0.9% and 3.6%, respectively, in 111 healthy men undergoing routine physical examinations. Thus previous exposure to hepatitis B virus (HBV) was estimated to be 8.8 times greater for homosexual men than for heterosexual men. Four of four HBsAg positive sera from homosexual men were subtyped as 'ad', whereas subtype 'ay' is preponderant in intravenous drug abusers. Future public health measures to control HBV infection should be directed to the prevention of sexually transmitted HBV infection among homosexual men.

Authors' summary

Sexually transmitted enteric pathogens in a male homosexual population

D. C. WILLIAM, Y. M. FELMAN, J. S. MARR, AND H. B. SHOOKHOFF (1978). *New York State Journal of Medicine*, **71**, 2050-2052

A review is made of reported cases of faecal-oral transmission of enteric patho-

gens in male homosexuals attributed to anilingus and, less commonly, to fellatio. Difficulties in making diagnoses lie in the recognition of the male homosexual patient, of the asymptomatic carrier states, and of the symptoms mimicking functional bowel syndromes or mild ulcerative colitis. Casual cold stool examination is considered insensitive, and repeated tests of multiple fresh purged stools are recommended. The danger of homosexual food handlers and waiters who are carriers passing on infection to patrons of restaurants is pointed out.

In a series of 89 stools examined from 100 middle-class homosexuals *Giardia lamblia* was found in nine, *Entamoeba histolytica* in seven, and non-pathogenic protozoa in 14. The suggestion is made that in New York City the majority of cases of amoebiasis and viral hepatitis in some areas are homosexually transmitted. In the lower West Side (Greenwich Village) the case rate (1975) for hepatitis B in men and women was 23.9 and 4.7 per 100 000, respectively, and for hepatitis non-B 45.8 and 10.2 per 100 000, respectively. Furthermore, in two health areas with the greatest homosexual congregation, case rates in men for hepatitis B were 66.3 and 24.7 per 100 000, no cases occurring in women; case rates of hepatitis non-B were 119.4 and 148.2 as against 10.3 and 12.5 per 100 000 for women. In these areas men were 11 times more likely to contract hepatitis than women living in the same areas.

M. Waugh

The gay bowel syndrome: Clinico-pathologic correlation in 260 cases

H. L. KAZAL, N. SOHN, J. I. CARRASCO, J. G. ROBILOTTI, AND W. E. DELANEY (1976). *Annals of Clinical and Laboratory Science*, **6**, 184-192

The gay bowel syndrome: A review of colonic and rectal conditions in 200 male homosexuals

N. SOHN AND J. G. ROBILOTTI (1977). *American Journal of Gastroenterology*, **67**, 478-484

The 'gay bowel syndrome' is described as a recurrent constellation of colonic and rectal conditions presenting to proctologists which are by no means exclusive to homosexuals but epidemiologically appearing more frequently in that group. The clinical diagnoses in decreasing order of frequency included condylomata acuminata, haemorrhoids, nonspecific pro-

ctitis, anal fistula, perirectal abscess, anal fissure, amoebiasis, benign polyps, viral hepatitis, gonorrhoea, syphilis, anorectal trauma and foreign bodies, shigellosis, rectal ulcers, and lymphogranuloma venereum.

The difficulties in recognition for the nonvenereologist of these disorders are highlighted. In addition to screening for sexually transmitted disease, stool examinations for enteric pathogens, ova, and parasites, proctosigmoidoscopy and biopsy, and barium enema studies are recommended.

M. Waugh

Social injuries of the rectum

N. SOHN, M. A. WEINSTEIN, AND J. GONCHAR (1977). *American Journal of Surgery*, **134**, 611-612

The dangers of 'fist fornication', the practice of introducing the closed or clenched fist into the rectal ampulla, upper rectum, and sigmoid colon to achieve sexual gratification, are reviewed. In a series of 11 male homosexual patients, mean age 35, who had received injuries, six had mucosal lacerations presenting with bleeding confined to the rectum, and four presented with physical findings of intra-abdominal perforation of the colon requiring laparotomy. One patient had an extensive injury to the anorectal sphincter resulting in complete anal incontinence.

The authors investigated the incidence of the practice by means of a questionnaire. Of 60 homosexuals admitting the practice, it was found that it occurred in older, more experienced men who often used various drugs, amyl nitrite, marijuana, LSD, mescaline, and alcohol, before the act. This potentially dangerous habit may be beginning to occur in heterosexual couples as well.

M. Waugh

John Hunter's alleged syphilis

GEORGE QVIST (1977). *Annals of the Royal College of Surgeons of England*, **59**, 205-209

The allegation that John Hunter suffered from syphilis is challenged. It is suggested that he was the subject of non-luetic vascular disease, evidence for which may be found by a study of his symptoms and necropsy report. It is further suggested that John Hunter's famous inoculation experiment was performed

not on himself but on another subject. It is claimed that there is in fact no scientific evidence for attributing John Hunter's illness to syphilis, and it is urged that the stigma of this diagnosis should be expunged from his image.

Author's summary

Male urethritis in King County, Washington 1974-75. I. Incidence

J. L. GALE AND M. W. HINDS (1978). *American Journal of Public Health*, 68, 20-25

Male urethritis in King County, Washington 1974-75. II. Diagnosis and treatment

M. W. HINDS AND J. L. GALE (1978). *American Journal of Public Health*, 68, 26-30

The virus of hepatitis B, a new dimension in the diagnosis of sexually transmitted diseases

H. C. W. STRINGER, E. R. SMITH, AND A. C. STEWART (1978). *New Zealand Medical Journal*, 87, 44-47

Behçet's syndrome in 32 patients in Yorkshire

M. A. CHAMBERLAIN (1977). *Annals of the Rheumatic Diseases*, 36, 491-499

HLA antigens associated with Behçet's syndrome

F. ERSOY, A. I. BERKEL, T. FIRAT, AND H. KAZOKOGLU (1977). *Archives of Dermatology*, 113, 1720

Bowen's disease of genital areas

A. LAPULESCU AND A. H. MEHREGAN (1977). *Journal of Cutaneous Pathology*, 4, 266-274

Assessing clinical practice in genitourinary medicine

Editorial (1978). *British Medical Journal*, 1, 740

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*A man of stature—in all ways—Henry Miller occupied a large part in many people's lives and his death in 1976 left a large gap. Though a neurologist of world renown he was best known in the North-East, where he spent most of his life, and where, for the last eight years of it, he was Vice-Chancellor of the University of Newcastle upon Tyne. A cross-section of his many friends and colleagues have contributed their reminiscences to **Remembering Henry** and, as each person saw him from a different angle, those who did not know him will find that the whole man gradually comes to life. Professor John Walton has written the introduction, three of Henry Miller's best-known articles are included (writing was his first interest after neurology), and there is a bibliography of his prolific publications. A fitting tribute, **Remembering Henry** may fill some of the gaps for those who have stayed behind. Profits from the book will be given to the Henry Miller Memorial Appeal.*

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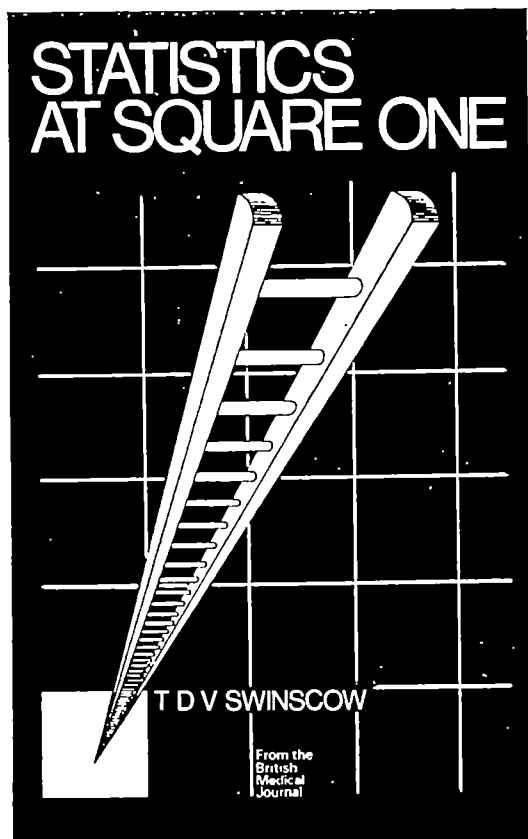
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A journal of the sexually transmitted diseases and allied disorders edited for the Medical Society for the Study of Venereal Diseases

Contents

Sexually transmitted diseases in Surinam

Observations and thoughts

H. E. MENKE (Surinam) *page 215*

Attachment of virulent *Treponema pallidum* to human mononuclear phagocytes

B. D. BRAUSE AND R. B. ROBERTS (USA) *page 218*

Lack of proliferation of circulating white blood cells in patients with syphilis before and after a Jarisch-Herxheimer reaction

M. HORSMANHEIMO (Finland) *page 225*

Lymphocyte reactivity in patients with gonococcal urethritis

L. ROSENTHAL AND E. SANDSTRÖM (Sweden) *page 229*

Genital inoculation of male *Macaca fascicularis* with *Neisseria gonorrhoeae* and *Ureaplasma urealyticum*

W. R. BOWIE, R. F. DIGIACOMO, K. K. HOLMES, AND J. L. GALE (USA) *page 235*

Effects of various divalent cations on the survival of *Neisseria gonorrhoeae* in liquid media

T. ODUGBEMI, M. MCENTEGART, AND S. HAFIZ (Gt Britain) *page 239*

Talampicillin and probenecid compared with ampicillin and probenecid for the treatment of gonococcal urethritis in men

S. AL-EGAILY, E. M. C. DUNLOP, P. RODIN, AND A. D. SETH (Gt Britain) *page 243*

Changes in the incidence of acute gonococcal and nongonococcal salpingitis

A five-year study from an urban area of central Sweden

L. FORSLIN, V. FALK, AND D. DANIELSSON (Sweden) *page 247*

Chlamydia trachomatis in non-specific urethritis

P. TERHO (Finland) *page 251*

Genital chlamydial infections in patients attending a gynaecological outpatient clinic

J. PAAVONEN, P. SAIKKU, E. VESTERINEN, B. MEYER, E. VARTAINEN, AND E. SAKSELA (Finland) *page 257*

Recurrent genital herpes among women: symptomatic v. asymptomatic viral shedding

M. C. RATTRAY, L. COREY, W. C. REEVES, L. A. VONTVER, AND K. K. HOLMES (USA) *page 262*

The value of a psychiatric service within the venereal disease clinic

S. BHANJI AND J. D. H. MAHONY (Gt Britain) *page 266*

Venereal disease education in West Virginia, USA

J. C. FRANZ AND R. J. WEISSER JR (USA) *page 269*

Urinary tract infection in a clinic for sexually transmitted diseases

M. G. MEAD AND R. N. GRÜNEBERG (Gt Britain) *page 274*

Early malignant syphilis observed during infection and reinfection in the same patient

K. LEJMAN AND Z. STARZYCKI (Poland) *page 278*

Correspondence *page 283*

Abstracts *page 285*

February 1979 Vol. 55 No. 1

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BRITISH MEDICAL ASSOCIATION TAVISTOCK SQUARE LONDON WC1

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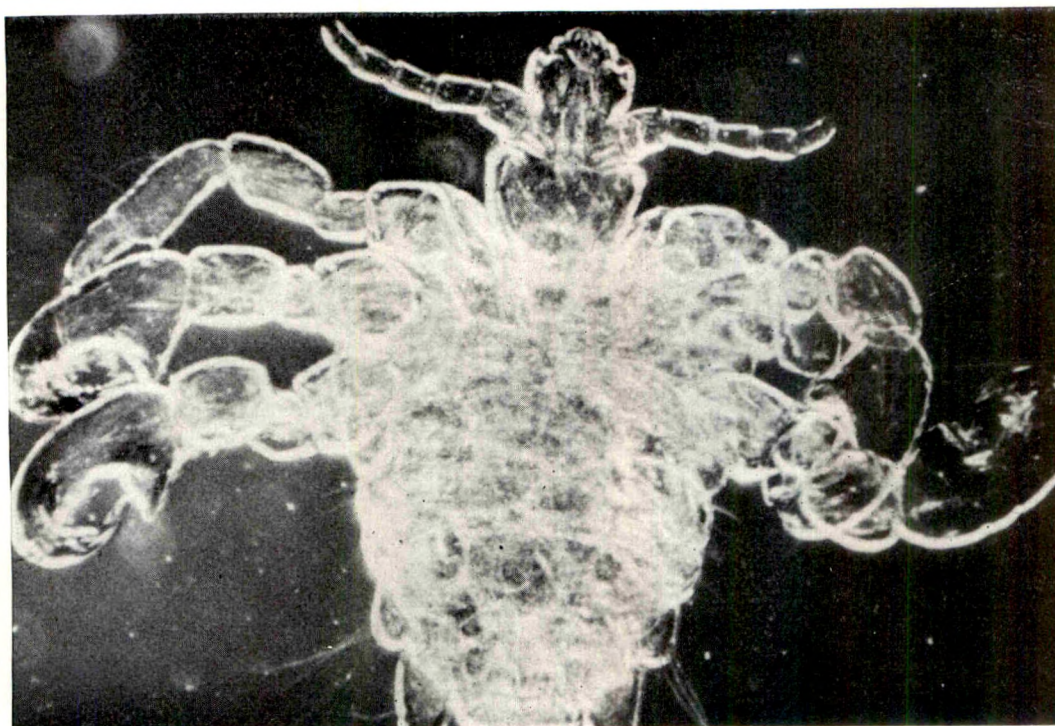
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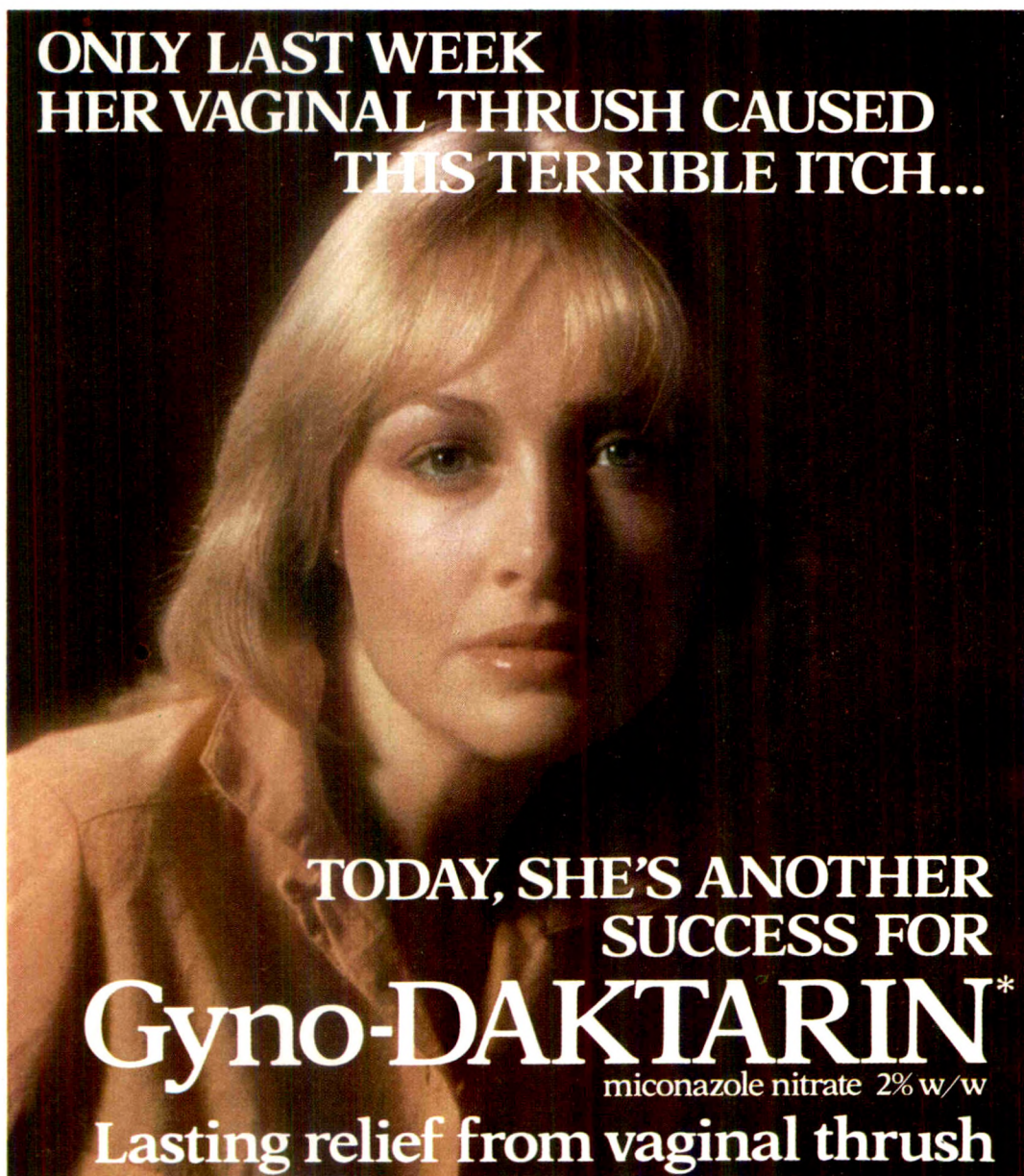
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1 Brit. J. Vener. Dis. (1975), 51, 97-98

2 Brit. J. Vener. Dis. (1975), 51, 206-209

3 Brit. J. Vener. Dis. (1976), 52, 43-45

4 Brit. J. Vener. Dis. (1978), 54, 107-111

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An assessment of the Microcult-GC culture test

P. F. UNSWORTH, H. TALSANIA, AND I. PHILLIPS

From the Department of Microbiology, St. Thomas' Hospital, London SE1

SUMMARY Microcult-GC, a miniaturised culture test, was compared with an established selective culture method for laboratory confirmation of gonorrhoea. Microcult-GC correlated 94% with the control method in identifying gonococci in male urethral specimens. Microcult-GC testing of 130 gonococci-negative vaginal swabs gave only 4.6% false-positive oxidase results, which could probably have been readily refuted by Microcult Gram stain. The test may be difficult to interpret for rectal specimens and is not appropriate for pharyngeal cultures.

Introduction

Many methods have been proposed for laboratory confirmation of the diagnosis of gonorrhoea. Microcult-GC is a miniaturised culture test for the presumptive identification of *Neisseria gonorrhoeae*, the performance of which requires minimal laboratory apparatus. Willcox and John (1976) made a preliminary study of the test and suggested it had potential for use in countries or areas lacking laboratory facilities and as a standard with which to compare other culture methods. We assessed the test in comparison with an established selective culture method (Phillips *et al.*, 1972) for its ability to detect *N. gonorrhoeae* in clinical material containing the organism and for its ability to yield negative results for vaginal and faecal material which was free of *N. gonorrhoeae*.

Methods

Specimens from five groups of patients were studied. Group 1 consisted of 50 men with Gram-negative diplococci in smears of urethral pus. Group 2 consisted of 18 women attending the sexually transmitted diseases (STD) clinic, of whom eight were sexual contacts of men with gonorrhoea or had themselves been previously infected with gonorrhoea, and 10 were new patients; both cervical and urethral swabs were examined. Group 3 consisted of 131 women of childbearing age (both inpatients and outpatients) in almost all of whom gonorrhoea was not suspected but from whom high

vaginal swabs were taken and sent to the laboratory. Group 4 consisted of 12 patients in whom gonorrhoea was not suspected but from whom faecal samples were collected and sent to the laboratory. Finally, group 5 consisted of a further eight men who conformed to the criteria for group 1.

Each specimen was inoculated in the STD clinic on to a Microcult-GC culture slide and also on to another medium. The latter consisted of Oxoid blood agar base no. 2 containing 10% lysed horse blood (LBA) for vaginal swabs from patients in group 3 and LBA with colistin methosulphate 100 000 u/l, vancomycin 3 mg/l, nystatin 12 500 u/l, and trimethoprim 5 mg/l (VCNT) (Phillips *et al.*, 1972) for samples from patients in other groups.

Microcult-GC culture slides were used according to the manufacturer's instructions. The slide and dessicant were carefully removed from each foil pouch just before use, and both culture areas of each slide were rehydrated with seven or eight drops of 4% v/v glycerol in water. The areas were immediately inoculated by one or other of two methods: in method A, which was used for specimens from patients in group 2, each of the two culture areas was inoculated with a specimen from a different site; in method B, which was used for specimens from patients in other groups, the same specimen was inoculated on to both culture areas in turn. Swabs were rolled over the culture areas to give as heavy an inoculum as possible. Each Microcult slide was replaced in its foil pouch together with a CO₂-generating tablet, the foil was sealed, and the slide then incubated at 37°C.

Microcult slides inoculated by method A were incubated for 42-48 hours; both areas were then processed. Slides inoculated by method B had one culture area processed after incubation for 18-24

Address for reprints: P. F. Unsworth, Cross-Infection Reference Laboratory, Central Public Health Laboratory, Colindale Avenue, London NW9 5HT

Received for publication 13 August 1978

hours and, if the first gave negative results, the other was processed after incubation for 42-48 hours. For 29 of the 50 urethral swabs from patients in group 1, the second culture area was processed whether or not the first gave negative results in the test. Incubation of the second culture area was prolonged to four or five days in the case of urethral swabs from patients in group 5.

At the end of the period of incubation slides were processed by three different methods in quick succession: firstly, an oxidase strip was applied for five seconds to the culture area and a positive reaction was recorded if part of the area turned blue within 30 seconds; secondly, the culture area was applied aseptically to a glass slide which was later Gram-stained and examined for Gram-negative diplococci (this was carried out for only nine specimens, none of which gave positive oxidase results, from group 3, but for all specimens from the other groups); and, thirdly, the whole area was applied aseptically to LBA for groups 1, 2, 3, and 5, and to VCNT for group 4 for subculture.

Subculture plates were incubated in 10% CO₂ in humidified air at 37°C, as were the plates on which specimens were inoculated in parallel with Microcult-GC. All plates were examined after incubation for 18-24 and 42-48 hours, and oxidase-positive colonies stained with anti-gonococcal fluorescent antibody (FA *N. gonorrhoeae*, Difco). Oxidase-positive organisms other than neisseriae cultured from specimens from patients in groups 3 and 4 were identified by conventional means.

Statistical comparisons were made by the χ^2 method.

Results

All oxidase-positive diplococci cultured in the study also showed specific immunofluorescence.

Detection of *N. gonorrhoeae* by means of Microcult-GC (method B) compared with culture on VCNT are shown in Table 1. Of 50 urethral swabs from men in group 1, 48 (96%) yielded positive results by culture on VCNT and a positive oxidase reaction by Microcult-GC. This was confirmed by Microcult-GC Gram stain in 45 (90%) and by the isolation of *N. gonorrhoeae* on subculture from Microcult-GC in all 48. The same two swabs gave negative results by VCNT culture and Microcult-GC. The correlation between Microcult-GC (method B) and VCNT culture for patients in group 1 was 94%.

The results of Microcult-GC (method A) compared with VCNT culture of urethral and cervical swabs from women in group 2 are given in Table 2. Of 18 cervical swabs six (33%) yielded gonococci on VCNT; by Microcult-GC three of these gave positive oxidase and Gram-stain results. Of 18 urethral swabs five (28%) yielded gonococci on VCNT; the Microcult-GC oxidase reaction was positive for four of the five and Microcult Gram-staining confirmed the presence of Gram-negative diplococci in three. Overall, subculture of Microcult-GC yielded *N. gonorrhoeae* from six out of 18 patients, from six cervical and four urethral swabs.

Table 3 refers to urethral swabs from 29 of the men in group 1 for whom a comparison was made of Microcult-GC results obtained by processing one Microcult-GC culture area after incubation for 18-24 hours or 42-48 hours or by combining the

Table 1 Results of Microcult-GC (method B) and culture on VCNT medium for urethral swabs from 50 men with gonorrhoea

Microcult-GC (method B)	Identification of gonococci			Culture on VCNT medium (positive result)
Oxidase reaction	+	+	-	
Gram stain	+	-	-	
Subculture	+	+	-	
No. of urethral swabs (%)	45 (90)	3 (6)	2 (4)	48 (96)

+ Positive - negative

Table 2 Results of Microcult-GC (method A) for specimens from 18 women attending a STD clinic*

Microcult-GC (method A)	Identification of gonococci from								
	Either swab			Urethral swab			Cervical swab		
Oxidase reaction	+	-	-	+	+	-	+	-	-
Gram stain	+	-	-	+	+	-	+	-	-
Subculture	+	+	-	+	+	-	+	+	-
No. of patients	4	2	12	3	1	14	3	3	12

+ Positive - negative

*VCNT culture detected gonococci in urethral swabs, cervical swabs, and either swab from 5, 6, and 6 patients respectively

Table 3 Effect of duration of incubation on Microcult-GC in 29 men with gonorrhoea*

Microcult-GC	Results after incubation of										One culture area for 18-24 h and one area for 42-48 h (method B)			
	One culture area for (hours)													
	18-24					42-48								
Oxidase reaction	+	+	-	-	-	+	+	+	+	-	-	+	+	-
Gram stain	+	-	+	-	-	+	+	-	-	+	-	+	-	-
Subculture	+	+	+	+	-	+	-	+	-	-	-	+	+	-
No. of urethral swabs	23	1	1	1	3	16	4	4	2	1	2	25	2	2

+ Positive - negative

*Urethral swabs yielded gonococci on VCNT medium after incubation for 18-24 h, 42-48 h, and for either period in 25, 2, and 27 patients respectively

two (method B). Method B yielded more positive results by oxidase reaction, Gram stain, and subculture than did processing one area after either 18-24 hours or 42-48 hours; similarly oxidase and Gram-stain results were more often both positive for the same specimen; differences were not significant ($P > 0.05$ in all comparisons). The Microcult-GC Gram stain showed red amorphous material in the absence of Gram-negative diplococci, which counted as a positive result, from five of 29 areas incubated for 42-48 hours, but from only one of 29 areas incubated for 18-24 hours.

All eight urethral swabs from patients in group 5 gave positive Microcult-GC oxidase, Gram-stain, and subculture results when processed after incubation for 18-24 hours. For three swabs the second Microcult culture area was processed after three days' incubation; the Gram stain of all three showed red amorphous material. In one case there were also occasional pairs of Gram-negative diplococci, and subculture of this specimen yielded gonococci. Subculture of the second specimen, and incubation for 48 hours, yielded two colonies of *N. gonorrhoeae*; and the third gave negative results on subculture. For five swabs the second Microcult culture area was processed after five days' incubation, at which time the Gram-stain results were: one positive, one negative, and three showing only red amorphous material. Subculture gave positive results only from the specimen with Gram-negative cocci in the Gram stain.

Of the 131 high vaginal swabs from patients in group 3 cultured directly on to lysed blood agar, nine yielded oxidase-positive organisms; six were pseudomonads and three were yeasts. By Microcult-GC (method B) seven swabs gave a positive oxidase reaction; Microcult-GC Gram staining was not performed on these, but subculture of one swab yielded *N. gonorrhoeae* and of the other six *Pseudomonas aeruginosa* or *Pseudomonas* species. The single case of gonorrhoea detected by Microcult-GC was not detected by culture on LBA, but gonococci

had been previously isolated from the patient by culture on VCNT.

Culture of 12 faecal specimens on VCNT yielded non-gonococcal oxidase-positive colonies in five cases. Microcult-GC (method B) yielded a positive oxidase reaction for two of them; *Pseudomonas aeruginosa* grew on subculture from one oxidase-positive specimen and a *Bacillus* species from the second.

Discussion

The principle of Microcult-GC is presumptive identification of *N. gonorrhoeae* by means of growth on selective medium, oxidase reaction, and Gram stain. The test would not distinguish either meningococci—though these occur only rarely in genital specimens (Faur *et al.*, 1975)—or other neisseriae, so that it could not be reliably used for pharyngeal specimens. Microcult-GC requires only sterile swabs, an incubator, Gram-staining equipment, and a microscope. Culture slides can be stored at room temperature ($< 30^{\circ}\text{C}$) for months.

A very faint blue tinge sometimes appeared within 30 seconds of applying the oxidase strip on culture areas not containing organisms, but with a little practice this was easily recognised as a negative reaction. Gram-staining of Microcult culture areas occasionally showed red amorphous material, apparently due to degenerate gonococci; its colour was identical to that of typically-stained gonococci and it was never seen in specimens with completely negative results. Prolonged incubation was associated with an increased frequency of red amorphous material and with decreased gonococcal viability on subculture. It is necessary to confirm positive oxidase results by Gram staining as various bacteria may produce these. Gram stains usually showed clear-cut Gram-positive and Gram-negative cocci and bacilli or the red amorphous material.

Unlike Williams *et al.* (1978) we found that subculture from Microcult-GC was easy and the results correlated completely with those of VCNT culture. Our method of subculture delivered a heavier inoculum and we minimised delay after oxidase-testing. Subculture could be used for biochemical or immunofluorescent tests and antimicrobial sensitivity-testing.

The rate of detection of gonococci from male urethral swabs by Microcult-GC was high; paired oxidase-positive and Gram-stain results were falsely negative in only 6% of cases. This could be remedied by subculturing all areas immediately after Gram staining, but this defeats the object of simplicity. Microcult-GC was less effective in detecting gonococci in female specimens but in these instances the inocula on Microcult may have been smaller than those on VCNT and the groups were too small for a complete assessment.

Microcult-GC false-positive oxidase reactions occurred with only 4.6% of high vaginal swabs. Microcult Gram staining would probably have given negative results for these specimens, which yielded pseudomonads on subculture. False-positive oxidase reactions were found for two out of 12 faecal specimens tested; one of these gave a negative result in the Microcult Gram stain, but the other result was difficult to interpret. Difficulties might be expected in using the Microcult test for rectal specimens, especially if there was heavy faecal contamination.

We agree with Willcox and John (1976) and Williams *et al.* (1978) that Microcult-GC may be useful in areas or countries which lack laboratory facilities. In addition, the test allows subculture of gonococci without the need to incorporate antibiotics into the medium used, which might be an advantage in some laboratories. The detection rate of gonococci in specimens with positive results from men is high and the false-positive rate for vaginal specimens is low. Our limited experience suggests that the method may be less sensitive for specimens from women with gonorrhoea and may yield results from faecally contaminated samples that are difficult to interpret.

We thank Dr C. Nicol, from whose patients swabs were obtained, and Mr I. Williams and Sister I. Rikof for help in obtaining these specimens. We thank Mr R. Newall of the Ames Company for kindly supplying Microcult-GC tests.

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Serum immunoglobulin response in uncomplicated gonorrhoea

ALEXANDER McMILLAN,* GILLIAN McNEILLAGE,† HUGH YOUNG,‡
AND SHEILA R. BAIN†

From the Department of Sexually Transmitted Diseases, Glasgow, and the Departments of †Venereology
and ‡Bacteriology, University of Edinburgh, Scotland

SUMMARY Sera from 225 men and 140 women were examined by an indirect immunofluorescent antibody technique for antibody reactive with *Neisseria gonorrhoeae*. Antigonococcal IgM was demonstrated at a titre of ≥ 16 in about 45% of infected, but in only 3% of non-infected, patients. Most of this antibody occurred in sera of patients who had been infected for less than 14 days. Antibody of the IgA class was found at a titre of ≥ 16 in over half the infected, but in none of the non-infected, patients. IgG antibody reactive with the gonococcus was found in each infected patient at a titre of ≥ 16 but in only 8% of controls. The mean log titre of this antibody was significantly higher in patients who had been infected for more than seven days than in those whose infection was of shorter duration.

Introduction

Although gonorrhoea is an infection usually confined to the mucosal surfaces of the body, a systemic antibody response may be demonstrated by various methods such as complement-fixation tests, haemagglutination reactions, and indirect immunofluorescent antibody techniques (Ratnatunga, 1971; Ward and Glynn, 1972; Welch and O'Reilly, 1973). The classes of antibody involved in this response have not, however, been clearly defined other than under experimental conditions (Cohen *et al.*, 1969).

It is the purpose of this paper to record the classes of immunoglobulins reactive with *Neisseria gonorrhoeae* which were found in the serum of patients with naturally acquired uncomplicated infection and to examine the effect of treatment.

Material and method

PATIENTS

Sera were obtained from patients (225 men and 140 women) attending the department of venereology at the Black Street Clinic, Glasgow.

Urethral gonorrhoea in men was diagnosed by microscopical examination of a Gram-stained smear

and confirmed by culture on selective medium. The identity of suspected colonies was confirmed by direct immunofluorescence and sugar fermentation reactions. Material from the urethra was taken for culture from all the male patients and where there had been homosexual contact specimens from the oropharynx and anorectal area were also taken for culture.

Gram-stained smears of urethral and cervical secretions were examined and culture specimens taken from these sites and from the oropharynx and anorectum in all female patients. Gonococcal infection was excluded only if three sets of cultures taken at weekly intervals from these sites gave negative results.

Neither those patients who had had antimicrobial treatment within the six weeks preceding attendance at the clinic nor those with complicated infection were included in the study.

ANTIGEN PREPARATIONS

As gonococcal antigen, strain 9 of *N. gonorrhoeae* as described by O'Reilly *et al.* (1973) was used (kindly supplied by Dr D. S. Kellog, US Center for Disease Control, Atlanta), as it had been shown to contain antigenic characteristics which were common to a variety of gonococcal strains but not shared by other *neisseriae*. The organism was cultured on a selective medium (Young, 1978). Cultures of *Neisseria meningitidis*, *Neisseria perflava*, *Neisseria lactamica*, and *Neisseria catarrhalis* for use in

Address for reprints: Dr A. McMillan, Department of Venereology, 67 Black Street, Glasgow, Scotland

Received for publication 21 August 1978

control studies were identified by fermentation reactions. They were obtained from stock cultures held in the Department of Microbiology at the University of Edinburgh.

Colonies were suspended in phosphate-buffered saline (PBS), pH 7.4, and the suspensions diluted until the fluid was faintly turbid. Aliquots were stored at -20°C until required.

A drop of suspension was placed in each well of a Multidot slide (Hendley, Essex, UK) and dried by incubation at 37°C for 10 minutes.

INDIRECT IMMUNOFLUORESCENT TEST

A standard indirect immunofluorescent technique (IF test) was used (Johnson *et al.*, 1978). Fluorescein-conjugated sheep anti-human IgA, IgG, and IgM sera were obtained from commercial sources (Wellcome Reagents, UK) and their specificity confirmed by gel immunodiffusion.

Doubling dilutions of sera, previously inactivated by heating at 56°C for 30 minutes, were prepared and layered on to the prepared slides. After the slides had been incubated at 37°C for 30 minutes and washed in PBS, conjugated antiserum at a dilution of 1/16 was added, and the slides maintained at 37°C for 30 minutes. The slides were then thoroughly washed in PBS and mounted in buffered glycerol (Difco).

Preparations were examined with a Zeiss microscope (Large Universal). After being scanned with

a low power objective detail was examined with a $\times 100$ oil immersion lens.

Fluorescence was graded according to the system used by Welch and O'Reilly (1973) as follows: 4+ indicated brilliant fluorescence of all organisms; 3+, well-defined fluorescence of all organisms in the field; 2+, low density fluorescence of at least 75% of organisms; and 1+, occasional fluorescing organisms. Only a 2+ fluorescence or higher reading was recorded as a positive result.

Statistical comparisons were made by the method of binomial probabilities and Student's *t* test.

Results

UNTREATED PATIENTS

Tables 1 and 2 show the results obtained in the indirect immunofluorescent antibody test applied to the serum from infected and non-infected men and women.

IgM

A titre of ≥ 16 of IgM reactive with *N. gonorrhoeae* was found in 44.8% (56/125) of men with untreated gonorrhoea but in only 3% (3/100) of non-infected men ($P < 0.001$ by the method of binomial probabilities). Similarly in 45.7% (32/70) of infected women and in 2.9% (2/70) of non-infected women antigenococcal antibody of this class was found at a titre of ≥ 16 ($P < 0.001$).

Table 1 *Immunoglobulin classes of antibody against Neisseria gonorrhoeae in sera from men with untreated gonorrhoea and from non-infected patients*

Patients	No. of sera	IgM titre					IgA titre					IgG titre				
		≤ 8	16	32	64	128	≤ 8	16	32	64	128	≤ 8	16	32	64	128
Infected																
For 1-7 days	52	19	19	9	5	0	19	14	12	6	1	0	4	13	18	17
8-14 days	45	25	11	8	1	0	17	12	13	3	0	0	0	0	10	35
15-21 days	14	11	3	0	0	0	12	1	1	0	0	0	0	0	4	10
22-28 days	2	2	0	0	0	0	1	1	0	0	0	0	0	0	1	1
≥ 29 days	12	12	0	0	0	0	12	0	0	0	0	0	0	0	4	8
Non-infected	100	97	3	0	0	0	100	0	0	0	0	92	8	0	0	0

Table 2 *Immunoglobulin classes of antibody against Neisseria gonorrhoeae in sera from women with untreated gonorrhoea and from non-infected patients*

Patients	No. of sera	IgM titre					IgA titre					IgG titre				
		≤ 8	16	32	64	128	≤ 8	16	32	64	128	≤ 8	16	32	64	128
Infected																
For 1-7 days	15	4	5	6	0	0	7	3	4	1	0	1	4	8	2	0
8-14 days	24	7	7	8	2	0	3	9	8	3	1	0	0	0	6	18
15-21 days	19	15	4	0	0	0	10	7	1	1	0	0	0	0	4	15
22-28 days	6	6	0	0	0	0	5	1	0	0	0	0	0	0	2	4
≥ 29 days	6	6	0	0	0	0	6	0	0	0	0	0	0	0	2	4
Non-infected	70	68	2	0	0	0	70	0	0	0	0	66	4	0	0	0

In 54.6% (53/97) of men who had been infected for 14 days or less but in 10.7% (3/28) who had been infected for longer the titre of antigenococcal IgM exceeded 8 ($P < 0.05$). The titre of IgM antibody was ≥ 16 in 71.8% (28/39) of women infected for less than 14 days but in only 12.9% (4/31) of women whose infection was of a longer duration.

IgA

Antibody of the IgA class reactive with the gonococcus was found at a titre of ≥ 16 in 51.2% (64/125) of men and in 55.7% (39/70) of women. Patients who were not infected (100 men and 70 women) had serum titres of ≤ 8 ($P < 0.001$).

When the infection had been present for 14 days or less antigenococcal IgA was found (at a titre of ≥ 16) in the serum of 62.9% (61/97) of men but in only 10.7% (3/28) of men who had been infected for longer ($P < 0.05$). Similarly, IgA antibody was found at a titre of ≥ 16 ($P < 0.05$) in 74.3% (29/39) of women who had had gonorrhoea for 14 days or less but in only 32.3% (10/31) of women whose infection was of longer duration.

IgG

Antibody of the IgG class reactive with the gonococcus was found at a titre of > 16 in the serum of all the 125 (100%) men with untreated gonorrhoea but in only 8% (8/100) of men who were not infected ($P < 0.001$). In women, this antibody was detected at a titre of > 16 in the serum of 98.6% (69/70) of infected patients and in 5.7% (4/70) of non-infected patients ($P < 0.001$).

The arithmetic mean of the log titre of IgG reactive with *N. gonorrhoeae* in the serum of men infected for seven days or less was 1.7830 and in patients who had been infected longer it was 2.0289. This is a statistically significant difference ($P < 0.001$ by Student's *t* test). Similarly, the arithmetic mean log titre of IgG antibody in women was 1.4249 and 2.4903 in the serum of women infected for seven days or less and for eight days or more respectively ($P < 0.001$).

Naturally occurring IgA reactive with other neisseriae

Table 3 shows the results obtained in the indirect immunofluorescent antibody test with monospecific IgA when applied to sera diluted to 1/16 from patients with gonorrhoea and from non-infected controls.

EFFECT OF TREATMENT ON CLASSES OF ANTIBODY

Table 4 shows the arithmetic mean log titre of antigenococcal antibodies before and at intervals after treatment. The decline in titre of antibodies of the IgM and IgA classes is shown as well as the much more gradual fall in IgG titre.

Discussion

Although indirect immunofluorescent antibody techniques using various antigen preparations have been evaluated as diagnostic tests (Welch and O'Reilly, 1973; Rodas and Ronald, 1974), there have been few studies correlating the distribution

Table 3 Results of the indirect immunofluorescent antibody test with monospecific anti-human IgA on sera of patients with gonorrhoea and of non-infected controls

Antigen	Patients' case no. (and sex)											
	Infected						Non-infected					
	1 (M)	2 (M)	3 (M)	4 (F)	5 (F)	6 (F)	7 (M)	8 (M)	9 (M)	10 (F)	11 (F)	12 (F)
<i>N. gonorrhoeae</i>	4+	4+	4+	4+	4+	4+	—	—	—	—	—	—
<i>N. meningitidis</i>	—	—	—	—	—	—	—	—	—	—	—	—
group A	—	—	—	—	—	—	—	—	—	—	—	—
group B	—	—	—	—	—	—	—	—	—	—	—	—
group C	—	—	—	—	—	—	—	—	—	—	—	—
group D	2+	2+	2+	—	2+	1+	—	—	2+	1+	—	—
group E	—	—	—	—	—	—	—	—	—	—	—	—
group 29E	—	—	—	—	—	—	—	—	—	—	—	—
group W135	—	—	—	—	—	—	2+	—	—	—	1+	2+
group X	—	1+	1+	1+	1+	—	1+	1+	1+	2+	—	2+
group Z	—	—	—	—	—	—	—	—	1+	—	—	—
<i>N. lactamica</i>	2+	—	2+	—	—	1+	1+	—	2+	1+	1+	1+
<i>N. perflava</i>	2+	1+	1+	1+	2+	3+	2+	2+	2+	2+	2+	2+
<i>N. catarrhalis</i>	1+	1+	1+	1+	2+	2+	1+	1+	1+	2+	1+	2+

1+ Occasional fluorescing organisms

2+ Low intensity fluorescence of all organisms

3+ Well-defined fluorescence of all organisms

4+ Brilliant fluorescence of all organisms

Table 4 Arithmetic mean of log titres of antibodies reactive with *Neisseria gonorrhoeae* in the serum of infected patients before and after successful treatment

	No. of patients	Immunoglobulin class		
		IgM	IgA	IgG
Before treatment				
Men	125	1.108	1.170	1.927
Women	70	1.118	1.183	1.901
7 days after treatment				
Men	62	1.039	1.131	1.869
Women	43	1.120	1.071	1.904
14 days after treatment				
Men	48	0.947	1.029	2.157
Women	41	0.977	1.057	1.894
28 days after treatment				
Men	45	0.903	0.917	1.772
Women	39	0.903	0.911	1.860

of antigenococcal antibodies within the immunoglobulin classes with the duration of infection.

In the present study antibody of the IgM class reactive with *N. gonorrhoeae* was detected at a titre of >16 in 45% of infected men and in 46% of infected women before treatment. The results obtained in women were similar to those recorded by Wilkinson (1975), who found this class of antigenococcal antibody at a titre of >16 in 43% of infected patients. He found similar antibody in only 32% of infected men; the duration of infection was not recorded.

IgM antibody was detectable in the serum of more than half of the men and of two-thirds of the women with untreated gonorrhoea who had been infected for less than two weeks. When infection was of longer duration this antibody was less commonly found at the lowest dilution of serum examined. This early IgM response was observed under experimental conditions by Cohen *et al.* (1969), who also showed that even without treatment those subjects who developed antigenococcal IgM had titres of this antibody which gradually fell over a period of about four months. Few patients in the present study who had been infected for more than 14 days had titres of antibody of >16 . Cohen *et al.* (1969), however, used inocula of organisms which were much larger than those which would be acquired in natural infection, and this may partly explain the more gradual decline in IgM activity which they had observed.

The antigenococcal IgA response to infection was similar to that of IgM. Antibody of this class reactive with *N. gonorrhoeae* was found in the serum of about two-thirds of men and of three-quarters of women who had been infected for less than 14 days. When the infection was of longer duration only about 11% of men and 32% of women had this antibody in the serum at a dilution of 1/16 or

greater. Similar findings of a rapid decline in serum IgA antibody activity within two to three weeks of acquisition of infection were reported by Cohen *et al.* (1969). The relationship between production of antigenococcal secretory IgA in the mucous membranes and the detection of serum IgA antibody will be dealt with elsewhere.

No naturally occurring IgA reactive with *N. gonorrhoeae* was detected, although natural antibody against *N. perflava* and *N. catarrhalis* was found in infected and non-infected patients as was, less commonly, antibody against *N. lactamica* and *N. meningitidis*, groups D, W135, and X.

Antigenococcal antibody of the IgG class was found in the serum at a dilution of 1/16 or greater of all infected men and of almost all infected women. The presumed false-positive rates were 8% and 6% respectively for men and women. In one study, where heat-labile antigen and antihuman IgG were used in a fluorescent antibody test, the false-positive rate was lower as was the sensitivity (Gaafar and D'Arcangelis, 1976).

IgG antibody activity increased throughout the first week of infection, so that by the second week most patients had antibody at high titres (>64). No obvious decline in antibody activity occurred in the subsequent two to three weeks. These results agree with those obtained by Cohen *et al.* (1969).

Successful treatment of infection in both sexes resulted in a rapid decline in antigenococcal IgM and IgA activity. A much more gradual decline in IgG antibody activity occurred. This presumably reflects differences in the half-life of the antibody classes (IgM, five days; IgA, six days; and IgG, 22 days [Tomasi, 1976]). This gradual decline in IgG antibody activity makes interpretation of diagnostic immunofluorescent test results difficult if the patient has recently been treated for gonorrhoea.

This research project was supported by a grant from the Scottish Home and Health Department (Research Grant no. K/MRS/36/C38), whose financial assistance is gratefully acknowledged.

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Diagnosis of gonorrhoea in women

R. NICOL THIN* AND ELIZABETH J. SHAW†

*From the Departments of *Genital Medicine and †Medical Microbiology,
St Bartholomew's Hospital, London*

SUMMARY The results of Gram stains and cultures from 145 women with uncomplicated ano-genital gonorrhoea are presented. The site which gave the highest yield of gonococci was the cervix. Equally good results were obtained with Stuart's transport medium and direct plating in the clinic. Positive results from rectal specimens alone were obtained from 8.4% of the 119 women in whom the rectum was examined. In 89 women the results of rectal specimens taken through a proctoscope were compared with those of swabs passed blindly up the anal canal. When Stuart's transport medium was used swabs taken through a proctoscope gave better results than the swabs taken blindly; the latter gave more positive results when direct plating was used. Thus when Stuart's medium is used a proctoscope is essential for the collection of rectal samples. Up to three sets of investigations were carried out where necessary, but 95% of the 145 cases were diagnosed on the results of the first set. However, only 54% of those patients diagnosed at their first visit had positive Gram-stain results. Further refinement of culture results is unlikely to improve diagnostic yield; what is needed is better, rapid diagnosis in the clinic.

Introduction

Women with uncomplicated gonorrhoea often have no symptoms or signs, and the diagnosis depends on the recognition of typical Gram-negative intracellular diplococci in stained smears and on the identification of *Neisseria gonorrhoeae* in cultures of secretions (King and Nicol, 1975). Other methods, such as serum antibody tests, still give too many false-positive and false-negative results to be of value in the diagnosis of individual cases (World Health Organisation, 1978). Even with modern laboratory methods the organism may be difficult to identify in infected secretions so that repeated examination may be necessary (Chipperfield and Catterall, 1976).

The object of this paper is to describe the findings at different sites in women with uncomplicated ano-genital gonorrhoea and the diagnosis rate at first and subsequent examinations

Material and methods

The findings in 145 consecutive female patients diagnosed as having gonorrhoea during 1976 and 1977 were assessed retrospectively.

CLINICAL INVESTIGATIONS

After routine history and genital examination the following investigations were undertaken. A bivalve speculum was inserted into the vagina and the ectocervix exposed. Vaginal secretion was wiped off with sterile cottonwool and material from the cervical os was collected with a sterile cottonwool bud; this was smeared on to a clean glass slide for Gram staining and a culture plate was inoculated (see below). A charcoal impregnated swab was inserted into the os and broken off in a bottle of Stuart's transport medium. The speculum was removed by running the upper blade along the anterior vaginal wall to massage secretions along the urethra to the meatus. A cotton bud was gently inserted into the meatus and a smear for Gram staining was made and a culture plate inoculated. The distal 2 cm of the urethra was then massaged through the anterior vaginal wall and a charcoal swab was inserted into the meatus and then placed in Stuart's transport medium. In the first 89 patients who presented as sexual contacts of men with gonorrhoea, a cotton bud was passed straight up the anal canal for approximately 5 cm until a relaxation was felt, suggesting the tip had reached the rectum, and was then withdrawn. A smear was made and a plate inoculated as before. A charcoal swab was then inserted in a similar manner and placed in Stuart's medium. These investigations will be called anal. Finally, a proctoscope was passed

Address for reprints: Dr R. N. Thin, Department of Genital Medicine, St Bartholomew's Hospital, London EC1A 7BE

Received for publication 8 September 1978

- .. and material for Gram stain, direct inoculation, and a Stuart's culture was taken. These investigations will be called rectal.

MICROBIOLOGICAL INVESTIGATIONS

Smears for Gram staining were prepared and read in the usual manner in the clinic.

Swabs were kept in Stuart's medium at room temperature for not more than 19 hours before plating on to the growth medium (Riddell and Buck, 1970). Plates inoculated in the clinic were immediately placed in a candle extinction jar, which was put into an incubator at 36°C in the clinic. After three to 18 hours the jar was transferred to the microbiology department where incubation was continued for a further 48 hours in a carbon dioxide incubator (Gallenkamp). Suspicious colonies were examined by the oxidase reaction and by sugar fermentation.

Yates's χ^2 test and the binomial test were used for statistical calculations.

Results

In all 145 cases samples from the urethra and cervix were examined, in 119 cases rectal samples were examined, and in 89 anal samples were examined. The overall findings obtained at each of the four sites are presented in Table 1. The highest positive yield was obtained from cervical samples and the lowest from anal samples. If only cervical samples for Gram stain and culture had been taken 85.5% of cases would have been diagnosed, whereas if only cervical culture specimens had been taken 79.3% of cases would have been diagnosed. If a

single cervical culture specimen had been taken at the patient's first visit 74.4% of cases would have been diagnosed.

Table 1 shows that culture specimens gave more positive findings than Gram stains at all sites, the difference being most marked in anal samples. Comparison of results of plates inoculated in the clinic and those inoculated with Stuart's swabs showed no significant difference ($P > 0.5$ in all combinations by the χ^2 test).

When cases giving positive results at only a single site (urethra, cervix, or rectum) are analysed (Table 2) the cervix gave the highest proportion, 18.6% of all cervical samples, the rectum the second highest (8.4%), and the urethra the third with 6.2% of positive results. Comparison of the urethral with rectal results shows no significant differences ($P > 0.1$ in all combinations); it is apparent that urethral Gram stains gave a poor yield, with only one case showing positive results by Gram stain and culture and two cases positive results by Gram stain alone. Examination of the findings at the urethra and cervix in Table 2 (too few rectal results for analysis) by the binomial test showed that smears and cultures gave similar results. If at the urethra the two positive Gram-stain results, unsupported by cultural findings, are excluded on the assumption that they are false-positives—as their admitted contacts did not have gonorrhoea (negative results to Gram stain and culture)—then the cultural findings are superior to the Gram stain findings ($P = 0.032$). In contrast, sexual contacts of seven of the nine patients with positive results to Gram-stain alone at the cervix did have culture-positive gonorrhoea.

Table 1 Positive results at each site sampled

Method	Urethra		Cervix		Rectum		Anus	
	No.	%	No.	%	No.	%	No.	%
Gram stain	24	16.5	80	55.2	18	15.1	2	2.2
Culture	82	56.5	115	79.3	62	52.1	15	16.8
Total examined	145		145		119		89	
Plate	28	56.0	37	74.0	21	42.0	8	23.5
Total plate cultures	50		50		50		34	

Table 2 Cases with positive results at one site only

Method	Urethra		Cervix		Rectum	
	No.	%	No.	%	No.	%
Positive results by						
Gram stain only	2	1.4	9	6.2	0	
Culture only	6	4.1	10	6.9	4	3.4
Gram stain and culture	1	0.7	8	5.5	6	5.0
Total	9/145	6.2	27/145	18.6	10/119	8.4

Table 3 Comparison of rectal and anal investigations in women (89 cases)

Rectal findings	Anal findings					
	Gram stain*		Culture†		Gram stain and culture‡	
	Positive	Negative	Positive	Negative	Positive	Negative
Gram stain						
Positive	2	8				
Negative	0	79				
Culture						
Positive			12	13		
Negative			3	61		
Gram stain and culture						
Positive					12	17
Negative					3	57

*Binomial test, $P=0.008$ †Binomial test, $P=0.022$ ‡Binomial test, $P=0.002$

There were few positive anal findings (Table 1). These are analysed in Table 3, which shows that rectal Gram-stain, rectal culture, and rectal Gram-stain or culture or both all gave significantly more positive results than the corresponding anal investigations. Had anal investigations been relied upon, only 15 positive results by Gram stain or culture or both would have been obtained compared with 29 positive rectal results. No case had positive results in anal investigations alone.

Finally, Table 4 shows that 95.2% of the patients were diagnosed as having gonorrhoea after one examination, 98.6% after two examinations, and the remaining 1.4% at the third examination. However, only 54.3% of those diagnosed at their first visit had positive Gram-stain results.

Table 4 Cases diagnosed at first, second, and third examinations

Examination	Positive results by			
	Gram stain or culture or both		Gram stain	
	No.	%	No.	%
First	138	95.2	75	54.3 (of 138)
Second	5	3.4	3	60.0 (of 5)
Third	2	1.4	2	100.0 (of 2)
Total	145	100.0		

Discussion

The results in Table 1 are in broad agreement with those of Barlow and Phillips (1978), although more of their cases had positive results at urethra and cervix. Comparison of results at one site only showed that Barlow and Phillips (1978) had fewer positive results by microscopy alone at the urethra (1 in 603) but more positive culture results, so the overall urethral results were similar. The same

pattern was evident in their cervical results. Barlow and Phillips (1978) suggested that urethral Gram stains, but not culture, might be omitted and the findings here lend support to this suggestion. Furthermore, it seems reasonable to suppose that the two patients in whom the urethral Gram-stain alone gave positive results did not, in fact, have gonorrhoea.

Previous comparisons of use of transport media and direct plating in the clinic have been contradictory. Danielsson and Johansson (1973) reported a 3% loss with Stuart's medium compared with direct plating, while Hosty *et al.* (1974) found a 44% loss with Stuart's medium. Our findings in Table 1 show no significant differences between the two methods and this will be reassuring to all those who have to use transport medium for geographical reasons (see, for example, Adler *et al.*, 1978).

Anal investigations gave a poor yield of positive results (15) compared with rectal investigations (29) (Table 3). This is why anal investigations were abandoned after the results from the first 89 patients had been analysed. These results agree closely with the findings of a similar study (Bhattacharyya and Jephcott, 1974), although it is not clear how far the swabs were inserted. In contrast, in a larger series (Deheragoda, 1977) similar results were obtained from anal swabs inserted the same distance as our own and rectal swabs passed through a proctoscope. Bhattacharyya and Jephcott (1974) used Stuart's transport medium while Deheragoda (1977) directly inoculated plates. In our small series of 34 cases in which direct plating was used, the yield of eight (23.5%) positive results was higher than in the larger series in which Stuart's transport medium was used (15 [16.7%] positive results in 89 cases) (Table 1).

While it is apparent that there is no difference between overall urethral and rectal results (Table 2) we believe that the finding of 8.4% of cases with

positive results in the rectum alone shows that examination of this site is important in the investigation of gonorrhoea in women, but when Stuart's transport medium is used, specimens must be collected through a proctoscope.

If a single cervical Stuart's culture had been taken at the first visit, 25.6% of cases would have been missed.

Our diagnosis rate for gonorrhoea, 95.2% at the first examination and 98.6% after two examinations (Table 4), is in broad agreement with other recent studies (Chipperfield and Catterall, 1976; Evans, 1976). Although Barlow *et al.* (1976) had a higher percentage of positive diagnoses (97%) at the first examination it seems unlikely that refinement of culture methods will produce great increases in diagnostic yield. What is more important is to consider methods for increasing accurate diagnosis in the clinic at the first visit, for this will continue to depend on identification of the gonococcus or gonococcal antigen (World Health Organisation, 1978). It is also apparent that accurate diagnosis of gonorrhoea will remain the concern of the specialist with appropriate microbiological support.

We wish to thank Jane Wadsworth of the Computing Unit for Medical Sciences, St Bartholomew's Hospital, for carrying out the statistical analyses.

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Pharyngeal colonisation by *Neisseria gonorrhoeae* and *Neisseria meningitidis* in black and white patients attending a venereal disease clinic

ROBERT C. NOBLE, ROBERT M. COOPER, AND BETTY R. MILLER*

From the Division of Infectious Diseases, University of Kentucky, College of Medicine, Lexington, Kentucky, USA

SUMMARY Pharyngeal colonisation by *Neisseria gonorrhoeae* and *Neisseria meningitidis* was studied in 2000 patients attending a venereal disease clinic. Of these patients, 64% were white and 36% were black. The incidence of gonococcal infections was highest in the period from June to August. The incidence of genital or rectal infections or both was higher in the black patients. Pharyngeal colonisation by gonococci was present in 1.3% of the patients. There were no significant associations between pharyngeal colonisation and the pharyngeal symptoms, race, sex, or marital state of the patients. Pharyngeal colonisation was more frequent in patients with gonococcal infections at other sites. However, in 40.7% of the patients with pharyngeal colonisation, the pharynx was the only culture-positive site. There was no significant difference in the auxotypes or in the antibiotic susceptibility of the pharyngeal and the rectal-genital isolates except in the susceptibility to spectinomycin. Our findings do not indicate that gonococci isolated from the pharynx differ significantly from gonococci isolated from rectal or genital sites. It was notable that meningococcal colonisation of the pharynx was significantly more frequent in the white patients. This may be a genetically determined phenomenon.

Introduction

Pharyngeal gonococcal infection is recognised as a form of asymptomatic gonorrhoea. It has been described in case studies and in selected groups of patients, such as homosexual men, pregnant women, and patients practising oro-genital intercourse (Fiumara *et al.*, 1967; Owen and Hill, 1972; Pariser, 1972; Rodin *et al.*, 1972; Courman *et al.*, 1974; Wallin, 1974; Shahidullah, 1976; Stutz *et al.*, 1976.) In the present study, pharyngeal culture specimens were taken from consecutive new patients attending a city-county venereal disease clinic. We examined the association of gonococcal pharyngeal colonisation and certain epidemiological variables in a patient population that was known to have a low frequency of oro-genital sexual contact (Noble *et al.*, 1977). Since the meningococcus, another

pathogenic *Neisseria* species, may colonise the same site, the throat cultures were examined for both pathogens by means of selective media. The meningococcal and gonococcal isolates were further examined by serological and microbiological methods to identify sub-populations that might be of epidemiological importance.

Materials and methods

STUDY POPULATION

The study population consisted of 2000 new patients attending the Lexington-Fayette County Venereal Disease Clinic from September 1975 to October 1976. Samples from the posterior pharynx were taken with sterile cotton-tipped swabs. Culture samples were also taken from the urethra in men and from the cervix and rectum in women. Race, age, sex, and marital state were recorded as part of the patient's routine history.

DIAGNOSTIC METHODS

Samples from the pharynx, genitals, and rectum were immediately streaked on modified Thayer-

*In co-operation with the Lexington-Fayette County Health Department

Address for reprints: Dr Robert C. Noble, Division of Infectious Diseases, Department of Medicine, University of Kentucky College of Medicine, Lexington, Kentucky 40506, USA

Received for publication 5 July 1978

Martin medium containing vancomycin, colistin, nystatin, and trimethoprim (Martin and Lester, 1971) and incubated at 36°C for 48 hours. The pharyngeal cultures were incubated in a candle extinction jar, and the genital and rectal cultures were incubated in an atmosphere containing CO₂. Patients with gonorrhoea were defined as those men having Gram-negative intracellular diplococci present in a urethral smear or those patients of either sex who had positive culture results on modified Thayer-Martin medium of an oxidase-positive (1% solution of N-N-dimethyl-p-phenylene-diamine monohydrochloride), Gram-negative diplococcus. The rectal-genital cultures used in this study were randomly selected from different patients attending the venereal disease clinic during the year of the study. The selection was performed so that the proportion of black and white patients in the rectal-genital group would be the same as in the pharyngeal group.

All pharyngeal and selected genital cultures were identified by bacterial morphology, Gram stain, oxidase reaction, and production of acid with one or more of the following sugars: glucose, maltose, lactose, sucrose, or fructose. The latter medium contained 1% of the test carbohydrate in cystine tryptic agar (Difco, Detroit, Michigan). GC medium base (Difco) with 1% IsovitaleX supplement (Baltimore Biological Laboratories, Cockeysville, Maryland) was used as the culture medium for bacterial isolation. Meningococcal serogrouping was performed by a slide agglutination technique (Feldman, 1970) with antisera (supplied by the US Center for Disease Control) for serogroups A, B, C, D, X, Y, and Z. The following meningococci (from the American Type Culture Collection) were used as reference standards: *Neisseria meningitidis* serogroup A, ATCC 13077; serogroup B, ATCC 13090; serogroup C, ATCC 13102; and serogroup D, ATCC 13113. Meningococcal and gonococcal isolates were stored at -70° in tryptic soy broth (Difco) containing 13% glycerol.

AUXOTYPING OF GONOCOCCI

Isolates from the pharynx and selected genital isolates of *Neisseria gonorrhoeae* were auxotyped by the method of Catlin (1973). Auxotyping was performed twice on each isolate and gonococci of known auxotype were included as controls.

ANTIBIOTIC SENSITIVITY-TESTING OF GONOCOCCI

Antibiotic sensitivity-testing was performed by means of a plate dilution technique (Jaffe *et al.*, 1976). Duplicate inocula were made with a Steers

replicator (Steers *et al.*, 1959). Strains of *N. gonorrhoeae* (F18 and F29) and *Sarcina lutea* (F20) (supplied by the US Center for Disease Control) with known antibiotic susceptibilities were included as controls when gonococcal isolates were tested for their antibiotic susceptibilities. The antibiotics (US Pharmacopeial Convention Inc., Bethesda, Maryland) were tested in the following concentrations by means of twofold dilution in agar: penicillin (2-0-008 µg/ml); ampicillin (2-0-008 µg/ml); spectinomycin (100-0-39 µg/ml); and tetracycline (25-0-1 µg/ml).

STATISTICAL METHODS

Yates's χ^2 tests were used for categorical data, and the Mann-Whitney U test (two-tailed) was used in the analysis of the antibiotic sensitivity data.

Results

PATIENT POPULATION

Of the 64% of white patients and the 36% of black 60% were men and 40% were women in both racial groups; 77% were single, 11% married, and 9% divorced or separated. Their mean age was 20 years. Gonococcal infection of the cervix or rectum was present in 25% of the women and urethral infection was present in 30% of the men. Urethral infections were more common in black men (47.1%) than in white men (21.0%) ($\chi^2=86.93$, $P<0.001$). Cervical and rectal infections were more common in black women (35.2%) than in white women (19.0%) ($\chi^2=24.7$, $P<0.001$).

PHARYNGEAL GONOCOCCAL COLONISATION

N. gonorrhoeae was isolated from the pharynx in 1.3% (27/2000) of the patients. None of the patients complained of a sore throat. Of the 27 patients with positive pharyngeal culture results for *N. gonorrhoeae*, 15 were white and six were black men, and three were white and three were black women. Pharyngeal gonococcal colonisation occurred with similar frequency in black (1.2%) and white (1.5%) patients. Although pharyngeal gonococcal colonisation was more than twice as common in men (1.8%) than in women (0.7%) this difference was not statistically significant. Pharyngeal colonisation was more common in patients with gonococci isolated from other sites, (2.8%, 16/570) than in patients who were otherwise free of gonococci (0.8%, 11/1430) ($\chi^2=11.22$, $P<0.001$). In patients with concurrent genital or rectal gonorrhoea, 3.3% (12/363) of the men and 1.9% (4/207) of the women had pharyngeal gonococcal colonisation. The pharynx was the only culture-positive site in 1.9% of

patients with gonorrhoea. Nine out of 21 men with pharyngeal gonorrhoea had negative urethral culture results, and two of six women with pharyngeal gonorrhoea had negative cervical and rectal culture results. Thus, in 40.7% (11/27) of patients with pharyngeal gonorrhoea, the pharynx was the only culture-positive site. Nineteen out of 21 men with pharyngeal gonorrhoea were asked about their sexual habits; only three stated that they were homosexual. Table 1 shows that during the study period the incidence of gonorrhoea was highest during the three months June to August ($\chi^2=27.08$, $P<0.01$). Gonococci were also more frequently isolated in pharyngeal cultures from patients during this period, and the increased frequency paralleled the increase in gonococcal isolates from genital and rectal sites.

Six different auxotypes were present among 26 of the pharyngeal isolates. One isolate became non-viable before auxotyping. The auxotypes of the pharyngeal isolates were compared with the auxotypes of 150 randomly picked rectal or genital isolates from different patients attending the venereal disease clinic during the year of the study.

The rectal-genital isolates were selected to represent the same proportion of black and white patients as were present in the pharyngeal group. Table 2 indicates no significant difference in the distribution of the auxotypes in the pharyngeal compared with the rectal-genital group. The pharyngeal and genital isolates were tested for their susceptibility to four antibiotics: penicillin, ampicillin, tetracycline, and spectinomycin. Of the antibiotics tested only the susceptibility to spectinomycin differed significantly between the two groups. As shown in Table 3, the pharyngeal isolates were more susceptible to spectinomycin than were the rectal-genital isolates.

PHARYNGEAL MENINGOCOCCAL COLONISATION

Meningococci were cultured from the pharynx in 9.5% of the patients. Meningococcal colonisation was more common in white (13.5%) than in black (2.3%) patients ($\chi^2=65.8$, $P<0.001$). Meningococcal colonisation was present in 15.3% of white men and in 12.4% of white women ($\chi^2=4.37$, $P<0.05$) compared with 2.4% of black men and

Table 1 Seasonal variation in percentage of patients with gonococcal infections

Infected site	Months				Total numbers
	Dec-Feb* (%)	Mar-May* (%)	June-Aug* (%)	Sep-Nov* (%)	
Pharynx only	18.2	9.1	45.4	27.3	11
Pharynx plus rectum or genitals or both	6.2	6.2	68.8	18.8	16
Rectum or genitals or both	20.8	16.2	37.5	25.4	554
Patients attending the clinic (%)	23.3	14.6	32.3	29.7	1997

$\chi^2=27.08$, $P<0.01$

*Percentage of total

Table 2 Comparison of auxotypes of *Neisseria gonorrhoeae* from pharyngeal and rectal-genital sources

Site	Auxotypes					Total number
	Zero (%)	Pro. (%)	Arg. (%)	AHU (%)	Other (%)	
Pharynx	30.8	42.3	7.7	15.4	3.8	26
Rectum/genitals	25.3	39.3	8.0	22.7	4.6	150

$\chi^2=4.46$, $P<0.5$

Table 3 Spectinomycin sensitivities of pharyngeal and rectal-genital isolates of *Neisseria gonorrhoeae* (%)

Site	Spectinomycin ($\mu\text{g/ml}$)							Total number
	25	12.5	6.25	3.12	1.56	0.78	0.39	
Pharynx	0	33.3	33.3	12.5	12.5	8.3	0	24
Rectum/genitals	1.4	52.5	25.2	13.7	4.3	1.4	1.4	139

$Z=2.057$, $P=0.0202$ (Mann-Whitney U test)

2.5% of black women. Meningococcal colonisation was not associated with marital state and no significant seasonal variation in incidence was noted. Meningococci were concurrently isolated from only two of the 27 patients who were colonised with gonococci in the pharynx. Meningococcal colonisation was also not associated with the presence of rectal or genital gonococcal infection. Of the 189 meningococcal isolates 148 were available for serogrouping; of these, 26% were autoagglutinable and 19% could not be grouped (Table 4).

OTHER NEISSERIA SPECIES

Sixteen isolates of other *Neisseria* species were identified in the pharyngeal cultures; nine (0.4%) were *N. lactamica*, five (0.2%) *N. flavescens*, and two (0.1%) *N. subflava*. None was isolated in association with gonococci or meningococci.

Discussion

The results of our study are compared with those of previous surveys of gonococcal pharyngeal colonisation in patients attending venereal disease clinics in the United States and other countries in Table 5. In contrast to the other studies, pharyngeal colonisation by gonococci in our patients was more common in men than in women. This difference might have been explained if there had been a large number of homosexual men among the clinic patients (Wiesner *et al.*, 1973). However, the majority of men with positive pharyngeal culture results said that they

were heterosexual, and case histories from these men suggested that the most likely route of infection was by oro-genital sexual contact. Pharyngeal colonisation by gonococci was equally frequent in patients of both races. This finding was unexpected since oro-genital with penile-vaginal sexual contact in patients from this clinic was previously shown to be significantly more frequent among white patients than black—17% of white women compared with 4% of black women and 17% of white men compared with 2% of black men (Noble *et al.*, 1977). Although histories of sexual contact were not obtained from all patients in the present study, the higher pharyngeal colonisation rate in men may be a reflection of the higher incidence of genital infection in men and of the predominance of white men in the clinic population.

Although an association between race and disease may be confounded with socioeconomic variables, black individuals may be more susceptible to the gonococcus and thus have a higher incidence of infection following exposure. Such a susceptibility could be mediated by a genetically determined biological mechanism. The present and other studies (Noble *et al.*, 1977; Brooks *et al.*, 1978) have shown that genital or rectal gonorrhoea is more common among black than white clinic patients. The present study also showed a marked difference in meningococcal colonisation of the pharynx in black patients compared with white. Race, as a factor in meningococcal colonisation, has not been extensively studied except in military recruits and

Table 4 *Distribution by race of meningococcal serogroups isolated from clinic patients (%)*

Race	Serogroup							Autoagglutinated	Non-groupable	Total number
	A	B	C	D	X	Y	Z			
White	4.4	18.4	6.6	0	2.9	14.7	6.6	27.2	19.1	136
Black	16.7	33.3	0	0	0	8.3	0	16.7	25.0	12

Table 5 *Studies of gonococcal pharyngeal colonisation (GPC) in venereal disease clinic patients with gonorrhoea*

Author	Date	Country of study	Total number of patients with gonorrhoea	% with pharyngeal colonisation		% of patients with GPC with pharynx as only positive site	% of GPC in clinic population
				Men	Women		
Bro-Jørgensen and Jensen	1971	Denmark	161	6	9.1	0	2.8
Weisner <i>et al.</i>	1973	USA (Seattle)	405	3.2	10.3	4.2	5.6
Bro-Jørgensen and Jensen	1973	Denmark	1104	7.0	10.0	14.5	?
Ødegaard and Gundersen	1973	Norway	1440	5.0	11.3	16.0	2.9
Stolz and Schuller	1974	Netherlands	1133	3.6	8.4	3.3	1.6
Hallqvist and Lindgren	1975	Sweden	3271	3.6	5.8	1.1	?
Newnham <i>et al.</i>	1975	Australia	122	6.2	11.1	36.0	1.1
Noble <i>et al.</i> (present study)	1979	USA (Lexington)	575	5.6	2.9	40.7	1.3

servicemen undergoing training. In one study, a small subgroup of black servicemen had positive meningococcal pharyngeal culture results in proportion to their numbers in the group (Farrell and Dahl, 1966). In another study, Polynesian soldiers had higher meningococcal colonisation rates than did European soldiers (Knights, 1972). However, meningococcal acquisition and colonisation among military recruits may be higher than in the general population. Our findings of a low meningococcal colonisation rate in black patients might be explained if they acquired meningococcal infections at an earlier age with a resulting immunity as adults. However, the incidence of meningococcal meningitis is the same or slightly higher among white compared with black children, whereas the incidence of pneumococcal and haemophilus meningitis is higher in black children (Fraser *et al.*, 1973; Floyd *et al.*, 1974). Racial differences have been described in the pharyngeal colonisation of another pathogenic micro-organism. Pharyngeal colonisation by *Staphylococcus aureus* but not β -haemolytic streptococci is significantly more common in white than in black patients (Noble, 1974). Unfortunately, the small number of meningococcal isolates from black patients in our clinic was inadequate to correlate the meningococcal serogroups with the patients' race. Thus, it is unknown if certain meningococcal serogroups are found more frequently in white compared with black individuals. Although this and other studies have shown a slightly higher colonisation rate in men than in women (Greenfield *et al.*, 1971), the acquisition rate of meningococcal pharyngeal colonisation is the same with respect to sex in military recruits (Melton *et al.*, 1977).

Willcox *et al.* (1977) obtained pharyngeal cultures from patients attending a venereal disease clinic and found that patients harbouring meningococci in the pharynx were more likely to have genital gonococci than those who were not carrying meningococci. We were unable to identify an association between pharyngeal meningococcal colonisation and genital infection with gonococci. The difference in the two studies may lie in the fact that in the present study all new clinic patients had culture specimens taken whereas in their study only selected patient groups had specimens taken for culture.

Gonococci isolated from the pharynx and from a second site in the same patient usually have identical antibiotic sensitivities (Bro-Jørgensen and Jensen, 1973; Stolz and Schuller, 1974; Hallqvist and Lindgren, 1975; Catlin and Pace, 1977a). However, pharyngeal isolates have been reported to be more resistant to ampicillin and penicillin (Ödegaard

and Gundersen, 1973; Hallqvist and Lindgren, 1975; Catlin and Pace, 1977b) and to have different auxotypes (Catlin and Pace, 1977b) when compared with genital isolates from other patients. Gonococcal strains requiring arginine, hypoxanthine, and uracil are more susceptible to penicillin G (Knapp and Holmes, 1975), and they are isolated more frequently from white patients (Crawford *et al.*, 1977). Thus, when gonococci from the pharynx are compared with gonococci isolated from other sites the race of the infected patient is a significant variable. In the present study the number of white and black patients was proportional in both groups and no significant difference was observed in the auxotype or in the susceptibility to the antibiotics, penicillin, ampicillin, or tetracycline. However, the pharyngeal isolates were more susceptible to spectinomycin than were the rectal-genital isolates. This finding is unexplained and is of no clinical value since spectinomycin is relatively ineffective in the treatment of pharyngeal gonococcal infections (Wiesner *et al.*, 1973). Thus, with this exception, our findings indicate no significant differences in isolates from the pharynx compared with isolates from rectal or genital sites.

The pharynx was the only culture-positive site in two-fifths of our patients with gonococcal pharyngeal colonisation. This represents less than 1% of cultures from all patients and less than 2% of cultures from all clinic patients with gonorrhoea. Therefore, the relatively low incidence of pharyngeal gonococcal infection coupled with the difficulty of performing large numbers of sugar fermentation reactions on pharyngeal isolates argues against the routine screening of all patients for gonococcal pharyngeal colonisation. The effectiveness of alternative diagnostic techniques should be evaluated to limit the number of cultures required and also to establish the consequences of untreated pharyngeal colonisation with gonococci. The finding of an increased incidence of meningococcal colonisation in white patients may indicate differences in genetically determined mucosal receptor sites in the pharynx and merits further study.

The authors gratefully acknowledge the co-operation of Dr Philip G. Weiler, Commissioner of Health, Lexington-Fayette County Health Department, and the staff of the Venereal Disease Clinic of the Health Department. We also thank Dr G. W. Somes, Department of Community Medicine, for his help in the statistical analysis.

The meningococcus grouping antisera were donated by Dr W. K. Harrell of the Biologic Reagents Section, US Center for Disease Control, Atlanta, Georgia, and Dr Max D. Moody, Technical

Director, Burroughs-Wellcome Co., Research Triangle Park, North Carolina. Strains of *Neisseria gonorrhoeae* (F18 and F29) and *Sarcina lutea* (F20) were provided by Dr Clyde Thornsberry, US Center for Disease Control.

Funds for this study were provided in part by research grant AI 11304 from the National Institute of Allergy and Infectious Disease and a grant from the Alexandrine and Alexander Sinsheimer Fund.

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Inhibition of *Neisseria gonorrhoeae* by normal human saliva

A. MELLERSH, A. CLARK, AND S. HAFIZ

From the Department of Medical Microbiology, University of Sheffield Medical School, Sheffield

SUMMARY Saliva was found to be a powerful and specific inhibitor of *Neisseria gonorrhoeae*. Although 28 other species of bacteria were tested, including *Neisseria meningitidis*, *Neisseria pharyngis* var *flava*, *Neisseria lactamica*, and *Neisseria catarrhalis*, we failed to find any others sensitive to saliva under similar conditions. The physical properties of the inhibitory substance indicated that it might be salivary α -amylase. To test this hypothesis α -amylase was extracted from saliva and was shown to have a high antigenococcal activity. Hog pancreas α -amylase also showed strong antigenococcal activity, thus the observations indicate that for the strains we tested α -amylase is inhibitory to gonococci. This observation indicates that either the gonococcal outer cell wall contains some unique lipopolysaccharides or that the gonococcus is unusually dependent on the integrity of these moieties. Whichever speculation proves to be true it indicates a need for a careful study of the gonococcal cell wall.

Introduction

Gonococcal infection of the pharynx is becoming increasingly common. It is usually asymptomatic and may be resistant to normal treatment regimens, since results of tonsillar swabs remain positive after those from other sites have become negative (Wiesner, 1975). Many clinicians believe that undiagnosed or resistant pharyngeal carriage presents an important residual pool of infection. In Denmark, Bro-Jørgensen and Jensen (1973) showed that in 10% of women with cervical gonorrhoea the organism could also be isolated from tonsillar swabs.

It has been known for many years that the gonococcus can infect the pharynx. However, until now, the effect of saliva on the organism has not been examined. Gordon (1916) tested the effect of sterile saliva on the meningococcus and found no inhibition of growth. Bibby *et al.* (1938) reported the action of saliva on 169 strains of bacteria but unfortunately did not include the gonococcus among these.

This study was first undertaken to examine the effects of saliva on *Neisseria gonorrhoeae*. The

inhibitory effect, which was consistently observed, was then further studied in order to characterise it and determine its nature.

Material and methods

SALIVA SPECIMENS

A total of 65 specimens of whole mouth unstimulated saliva was studied. Of these, 35 specimens were obtained from patients attending the Sheffield special treatment clinic and 30 were from healthy controls; the age range was from 20 to 60 years. Pure parotid saliva was obtained from one individual by means of a Curby cup placed over the parotid duct. A pool of saliva for more detailed analysis was collected from one individual. All saliva samples were sterilised by Seitz filtration.

BACTERIA STUDIED

Neisseria gonorrhoeae

A total of 13 strains of *N. gonorrhoeae* was studied; five were international strains F62, K2, and WHO strains III, V, and VII; three were β -lactamase-producing strains, HK1, L6, L7 (kindly provided by Dr A. Percival) (HK1 was the strain selected to test all saliva specimens and to study the effect of saliva in detail); and the other five were fresh isolates from the throats of women attending the Sheffield special treatment clinic, designated R34, R37, R38, R40,

Address for reprints: A. Mellersh, Department of Medical Microbiology, University of Sheffield Medical School, Beech Hill Road, Sheffield S10 2RX

Received for publication 15 August 1978

- and R44. All strains were preserved by suspension in 1% proteose-peptone broth with 8% glycerol and were stored in the vapour phase of liquid nitrogen.

Other bacteria

- The other species of *Neisseria* studied are as follows:

Gram-positive cocci

Staphylococcus Oxford
Staphylococcus 80/81
Micrococcus luteus NCIB 9278
Streptococcus group A
Streptococcus group D
Streptococcus viridans
Diplococcus pneumoniae
Sarcinia

Gram-negative cocci

Neisseria meningitidis
Neisseria catarrhalis
Neisseria pharyngis var *flavus* NCTC 4590
Neisseria lactamica

Gram-positive bacilli

- *Corynebacterium xerosis*
- Listeria monocytogenes*
- Lactobacillus plantarum* NCIB 6376
- Bacillus subtilis*
- *Bacillus cereus*
- Clostridium welchii*
- Clostridium septicum*

Gram-negative bacilli

Escherichia coli
Shigella sonnei
Salmonella typhimurium
Proteus mirabilis
Klebsiella pneumoniae
Pseudomonas aeruginosa
Haemophilus influenzae
Bacteroides melaninogenicus
Bacteroides fragilis

No inhibition was demonstrated.

CULTURE AND INHIBITION OF GROWTH BY SALIVA

- The solid medium used for *N. gonorrhoeae* was Difco GC base plus 2% defined supplement (L-glutamine 1.0 g, dextrose 40.0 g, ferric nitrate 0.05 g, and cocarboxylase 0.02 mg per 100 ml distilled water). When the other bacteria studied failed to grow on the GC medium other suitable

media were used. Thus, *Haemophilus influenzae* was tested on chocolate agar and *Bacteroides* species anaerobically on modified BM agar plus 5% lysed blood.

Inhibition of growth on solid medium was tested as follows. A well-plate technique was used with 4 mm wells cut in the solid medium in standard Petri dishes. Each well was filled with saliva, which was allowed to diffuse into the medium for four hours at 4°C, after which time any remaining saliva was removed with a Pasteur pipette. The Petri plates were then flooded with an inoculum of the test organism suspended in saline and containing approximately 10^7 organisms per ml and any excess was then removed. After incubation for 18 hours at 37°C in 10% CO₂ with raised humidity the purity of growth was checked by Gram stain and the degree of growth inhibition was recorded.

The liquid medium used for the growth of *N. gonorrhoeae* was ANM (Hafiz and McEntegart, 1976) plus 2% defined supplement. In order to measure the inhibitory effect of saliva an initial 1/10 dilution of sterile saliva in ANM medium was made and doubling dilutions prepared in the same medium to a final dilution of 1/40 960. Each of these dilutions was inoculated with approximately 10^8 organisms in 0.1 ml of ANM.

Clostridia species were tested on reinforced clostridial medium (Oxoid Ltd, 1976) plus 10% saliva and incubated anaerobically.

All cultures were incubated for 18 hours at 37°C and purity of growth confirmed by culture and microscopy.

SURVIVAL OF *N. GONORRHOEAE* IN SALIVA

A heavy suspension of gonococci containing approximately 5×10^7 organisms/ml was prepared in ANM and divided into two 2-ml aliquots; to one, an equal volume of fresh sterile saliva was added and, to the other, an equal volume of saliva that had been heated to 90°C for five minutes. Both specimens were kept at 37°C and aliquots were taken from each at intervals for culture in ANM and for examination by phase contrast microscopy.

FURTHER EXAMINATION OF SALIVA IN RELATION TO *N. GONORRHOEAE* (HK1)

The effect of saliva on the gonococcus was assayed before and after the following procedures.

Effect of heat

Aliquots of whole saliva were heated for five minutes at temperatures from 40°C to 100°C at 5°C intervals. The aliquots were also assayed for α -amylase activity (see below).

Ultra-violet light

Fresh sterile saliva in an open Petri dish was exposed to ultra-violet light for five minutes.

Dialysis

Saliva was dialysed against 100 volumes of sterile saline overnight at 4°C.

Ammonium sulphate precipitation

Saturated ammonium sulphate was added slowly to aliquots of saliva so that the final concentrations were 30, 40, 55, and 70% saturation. Precipitates were centrifuged and resuspended in sterile saline. The precipitates and supernatants were dialysed against two changes of saline to remove ammonium sulphate before they were tested for antigenococcal activity.

PURIFICATION OF SALIVARY α -AMYLASE

This was performed according to the method of Meyer *et al.* (1947).

 α -AMYLASE-ACTIVITY TEST

A starch solution of 5 mg/ml was prepared with 1% calcium lactate to replace any calcium ions that might have been lost during saliva purification. One millilitre of the saliva fraction was added to 5 ml of the substrate mixture and incubated at 56°C for 30 minutes. A saline control was incubated in parallel. The α -amylase activity was estimated visually after adding one drop of 1% iodine to test and control tubes.

Hog pancreas α -amylase and the purified salivary amylase were tested for antigenococcal activity by the well-plate technique.

FRACTIONATION OF ACTIVE ANTIGONOCOCCAL COMPONENT OF SALIVA

Whole mouth saliva was concentrated fifteenfold and 4 ml applied to a Sephadex G-75 column (Pharmacia, Uppsala, Sweden), 40 cm $\times$ 2.0 cm, equilibrated with phosphate-buffered saline (0.15 mol/l, pH 7.2). The saliva was eluted with the same buffer and the eluate monitored for absorption at 280 nm with a LKB Uvicord II. Fractions were assayed by the techniques described above. Subsequently, hog pancreas α -amylase and purified salivary α -amylase were fractionated with the same apparatus.

Results

On solid media growth of all 13 strains of gonococci was inhibited by whole mouth saliva in a zone 16–20 mm in diameter round the well. Pure parotid saliva produced slightly greater inhibition. Different strains of gonococci showed variation in their

ability to grow in the presence of different saliva concentrations in liquid media (Table) but no strains grew in media with a saliva concentration of 1/320 or greater. All strains isolated from the throats of patients were strongly inhibited by saliva. No preponderance of any particular Kellogg type was noticed at the edge of the zone of inhibition nor was there any apparent correlation between Kellogg types and inhibition of growth by saliva.

All other bacteria tested grew freely in the presence of saliva.

Saliva from all 65 individuals tested strongly inhibited the HK1 strain of *N. gonorrhoeae*.

In survival tests gonococci were viable up to six hours after incubation with normal saliva and for up to 12 hours after incubation with heated saliva. No bacterial lysis was observed by phase contrast microscopy.

The inhibitory factor in saliva was destroyed by heating at 75°C for five minutes but not at 70°C for the same length of time; loss of α -amylase activity occurred at the same temperature. Exposure to ultra-violet light for five minutes did not alter activity. The active factor was not dialysable but was precipitated when ammonium sulphate was added to a concentration of 40% saturation. From these observations it seemed possible that the active factor might be salivary amylase. Chromatography of whole saliva, hog pancreas α -amylase, and purified salivary α -amylase is illustrated in the Figure. There were two peaks of antigenococcal activity in whole saliva and one in the hog pancreas α -amylase and the purified salivary α -amylase. The whole saliva second peak of antigenococcal activity coincided with that of the salivary α -amylase while that of the hog pancreas α -amylase was of slightly higher molecular weight. All peaks of antigenococcal activity had strong α -amylase activity.

Purified salivary α -amylase and hog pancreas α -amylase both showed strong antigenococcal activity.

Discussion

From the observations we have reported we believe that salivary α -amylase is a powerful and selective inhibitor of *N. gonorrhoeae*.

Table Growth of gonococcus strains in liquid media containing different concentrations of saliva

Saliva concentration	Strains growing
$\geq 1/320$	—
$< 1/640$	K2, L6, L7, WHO VII, R38
$< 1/1280$	WHO III, WHO V, R34, R37, R40, R44
$< 1/12560$	F62, HK1

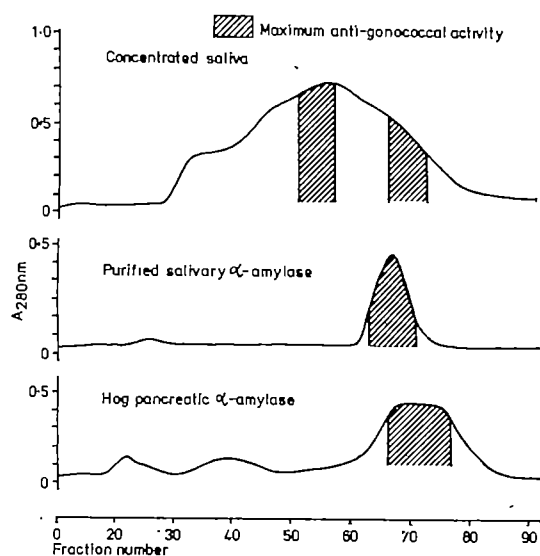


Figure 1. Tracings of whole-mouth saliva, hog pancreas α -amylase, and purified human salivary α -amylase gel-chromatographed on Sephadex G-75

The outer envelope of the gonococcal cell is generally believed to consist of three components, pili or fimbriae, protein subunits, and lipopolysaccharide units. Stead *et al.* (1975) compared the constituents of the cell wall lipopolysaccharides of commensal *Neisseria* species with those of gonococci and concluded that they were essentially similar.

If we assume that the α -amylase (approximately 50 000 Daltons) is acting on the outer cell wall and not passing into the gonococcus, a possible target for its action is the polysaccharide component of the lipopolysaccharide moieties. This may indicate

that either the structure of the gonococcal lipopolysaccharide is different from that of other *Neisseria* species (although the actual constituents may be the same) or the integrity of these moieties is especially important to the gonococcus. Possibly both mechanisms could account for the peculiar susceptibility of the gonococcus to α -amylase.

The authors are grateful to Professor M. G. McEntegart for his encouragement and help and to the staff of the Department of Medical Microbiology, University of Sheffield, for their assistance in this work. We are grateful to Dr A. Percival, Department of Medical Microbiology, University of Liverpool, for supplying some of the strains of gonococci and to the staff of the Special Treatment Clinic, Sheffield Royal Infirmary, for their co-operation and assistance.

A. Mellersh was supported by Difco on a student vacation fellowship.

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Short-term treatment of gonorrhoea with intramuscular and oral forms of trimethoprim-sulphamethoxazole

ALLAN LASSUS* AND OLLI-VEIKKO RENKONEN†

From the *Department of Dermatology and Venereology, University Central Hospital, Helsinki, and the

†Department of Immunology and Bacteriology, University of Helsinki, Finland

SUMMARY Fifty-nine male patients and 41 female patients with uncomplicated gonorrhoea were treated with an intramuscular dose of 1600 mg sulphamethoxazole plus 320 mg trimethoprim followed by an oral dose of 2400 mg sulphamethoxazole plus 480 mg trimethoprim 12 hours later. Forty-nine out of 52 male patients and 37 out of 38 female patients who were re-examined were cured. One of the men probably had a reinfection.

Introduction

Csonka and Knight (1967) reported favourable results with a combination of trimethoprim and sulphamethoxazole in the treatment of gonorrhoea. Later several studies confirmed these results. Recently an intramuscular form of trimethoprim-sulphamethoxazole has been developed which gives high blood concentrations of short duration. To obtain a high blood level for at least 24 hours we prescribed one intramuscular dose of trimethoprim-sulphamethoxazole and one oral dose 12 hours later to evaluate its effect on uncomplicated gonorrhoea in patients of both sexes.

Material and methods

The series included 59 male and 41 female patients with uncomplicated gonorrhoea. Specimens for direct microscopy and culture were taken from the urethra in men and from the urethra, cervix, and rectal mucosa in women before treatment and at the follow-up visit seven to 14 days later. The specimens for culture were transported in Stuart's medium to the laboratory and cultured on two Thayer-Martin media, one of which also contained trimethoprim. Although treatment was given on the basis of positive microscopical evidence, only cases confirmed by culture (including fermentation reac-

tions) were included in the assessment. Sensitivity tests to penicillin G and trimethoprim-sulphamethoxazole were carried out by the plate dilution method described by Reyn *et al.* (1958).

The treatment was given as an immediate intramuscular injection of two ampoules of Bactrim (Ro 6-2580/59, F. Hoffmann-La Roche and Co. AG, Basle, Switzerland) in equal doses into both gluteal regions, each ampoule containing 800 mg sulphamethoxazole and 160 mg trimethoprim.

Twelve hours later the patient was asked to take three tablets of Bactrim Quiksolv (Ro 6-2580/55, F. Hoffmann-La Roche and Co. AG, Basle, Switzerland) dissolved in a glass of water, each tablet containing 800 mg sulphamethoxazole and 160 mg trimethoprim.

Results

The MIC values for both penicillin G and trimethoprim-sulphamethoxazole were estimated in 93 cases (Table 1). In nine cases the gonococci showed a slightly decreased sensitivity to trimethoprim-sulphamethoxazole (MICs=1.25 and 25 µg/ml) and in 23 cases the MIC of penicillin G was ≥ 0.2 IU/ml. There seemed to be no cross-resistance between penicillin G and trimethoprim-sulphamethoxazole.

Ninety out of all 100 patients returned for re-examination, which showed that 49 (94.2%) of the 52 men and 37 (97.4%) of the 38 women had negative culture results (Table 2). One of the male patients probably had a reinfection because he

Address for reprints: Dr A. Lassus, Department of Dermatology and Venereology, University Central Hospital, Snellmanink 14, Helsinki 17, Finland

Received for publication 3 April 1978

Short-term treatment of gonorrhoea with intramuscular and oral forms of trimethoprim-sulphamethoxazole 25

Table 1 Sensitivity-testing of 93 gonococcal strains to penicillin G and trimethoprim-sulphamethoxazole

MICs of penicillin G (IU/ml)	MICs of trimethoprim-sulphamethoxazole (µg/ml)			Total
	0.31 and 5.3	0.62 and 12.5	1.25 and 25.0 (Slightly resistant)	
<0.1	35	24	2	61
0.1	5	3	1	9
0.2	7	2	2	11
0.5	4	4	3	11
1.0	0	0	0	0
2.0	0	0	1	1
Total	51	33	9	93

Table 2 Treatment results with trimethoprim-sulphamethoxazole given in a single intramuscular dose (1600 mg + 320 mg) and a single oral dose (2400 mg and 480 mg) 12 hours later

Patients	MICs of trimethoprim-sulphamethoxazole (µg/ml)			Total
	≤0.62 and 12.5	1.25 and 25.0	Not known	
Treated (59 men, 41 women)	84	9	7	100
Re-examined	77	8	5	90
Cured	76	5	5	86
Treatment failures	0	3	0	3
Reinfections	1	0	0	1

admitted sexual contact following treatment and harboured gonococci which were highly sensitive to trimethoprim-sulphamethoxazole. All the remaining three patients with positive culture results on re-examination had gonococci which were slightly resistant to trimethoprim-sulphamethoxazole, and they denied sexual contact following treatment. Thus, it seems probable that these patients (one woman and two men) were true treatment failures. Thus, of the 90 patients who were re-examined 3.3% were treatment failures. Three (33.3%) of the nine patients with a slightly decreased sensitivity to trimethoprim-sulphamethoxazole (MICs=1.25 and 25 µg/ml) were treatment failures.

The only side effect observed during the study was pain at the injection site in one patient; this disappeared after two days.

Discussion

The sensitivity-testing of the gonococci in the present study showed that fewer than 10% of the tested strains had a slightly decreased sensitivity to trimethoprim-sulphamethoxazole. The decreased sensitivity did not seem to cross-react with the decreased sensitivity to penicillin, which was about

25% in the present series. It seems, therefore, that trimethoprim-sulphamethoxazole is a good substitute in cases in which penicillin is ineffective.

It seems evident that the plate dilution technique for trimethoprim-sulphamethoxazole gives adequate information on the clinically valuable sensitivities of gonococci for trimethoprim-sulphamethoxazole, at least with the present treatment schedule. All treatment failures (three patients) were infected by strains with MIC values of 1.25 and 25 µg/ml for trimethoprim-sulphamethoxazole, but no cases of treatment failure occurred in the group of patients with more sensitive gonococci.

The side effects were minimal; no allergic rashes were seen and only one patient had severe pain at the injection site.

Thus, the one-day treatment schedule we used for cases of uncomplicated gonorrhoea seemed to be effective and safe in patients of both sexes.

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Antibodies to *Chlamydia trachomatis* in acute salpingitis

J. D. TREHARNE,* K. T. RIPA,† P.-A. MÅRDH,† L. SVENSSON,‡
L. WESTRÖM,‡ AND S. DAROUGAR*

From the *Virus Laboratory, WHO Collaborating Centre for Reference and Research on Trachoma and other Chlamydial Infections, Institute of Ophthalmology, London; the †Institute of Medical Microbiology, University of Lund; and the ‡Department of Obstetrics and Gynaecology, University Hospital, Lund, Sweden

SUMMARY Recent isolation studies have shown *Chlamydia trachomatis* to be an important aetiological agent in acute salpingitis in women. The present serological study indicates that *C. trachomatis* is the probable aetiological agent in two-thirds of 143 women with pelvic inflammatory disease (PID). In general, high levels of chlamydial antibody were found in sera and fluids aspirated from the pouch of Douglas and such antibody titres were shown to correlate with the severity of clinically graded tubal inflammation.

Introduction

The sequelae of acute salpingitis (pelvic inflammatory disease, PID) are the most common cause of infertility in women (Weström, 1975).

Several different micro-organisms have been implicated as the cause of PID, suggesting that the disease has a polymicrobial aetiology (Eschenbach *et al.*, 1975).

The vast majority of cases of acute salpingitis are caused by micro-organisms that have colonised or infected the cervix or both and reached the tubes as the result of an ascending infection (Falk, 1946; Danielsson *et al.*, 1975).

Although *Neisseria gonorrhoeae* has often been considered the major cause of this disease, Mårdh *et al.* (1977a) could recover the organism in only approximately one out of 10 patients with acute salpingitis and cervical gonorrhoea. Furthermore, recent culture studies have shown that over 30% of cases of PID may be due to *Chlamydia trachomatis* (Mårdh *et al.*, 1977b). In these patients with PID, who harboured chlamydiae in the cervix, the organism was recovered from the uterine tubes in six out of seven cases.

In the present study, in which we used the modified micro-immunofluorescence test (Treharne *et*

al., 1977a), we aim to assess the level and prevalence of different immunoglobulin classes of type specific chlamydial antibodies in sera and in fluid aspirated from the pouch of Douglas in a series of women with laparoscopically verified acute salpingitis and to present the findings in relation to the visual clinical grading of tubal inflammation.

Material and methods

PATIENTS

Since 1960, diagnostic laparoscopy has been performed on all patients with the presumptive diagnosis of acute salpingitis admitted to the department of obstetrics and gynaecology at the University Hospital, Lund, Sweden (Weström and Mårdh, 1977). This study included patients examined at this clinic during a period between 1973 and 1977.

Laparoscopy was carried out on the day of admission of each patient. The laparoscopic technique and the criteria of a laparoscopic diagnosis of acute salpingitis have been described in detail elsewhere (Jacobson and Weström, 1969). The inflammatory changes of the fallopian tubes, as seen at laparoscopy, were graded as mild (reddened serosa of the tubes, oedema of the tubal wall, and purulent discharge from the abdominal orifice), moderate (more advanced inflammatory changes confined to the tubes, such as tubal swelling, closure of the abdominal orifices, and fibrin deposits

Address for reprints: J. D. Treharne, Virus Laboratory, Institute of Ophthalmology, Judd Street, London WC1H 9QS

Received for publication 26 June 1978

on the serosal surfaces of the tubes), or severe (pelvic peritonitis or abscess formation).

COLLECTION OF SPECIMENS

Serum samples were collected on the day of admission from 143 women with acute salpingitis. During laparoscopy, peritoneal fluid from 27 patients was aspirated from the pouch of Douglas by means of a canula inserted through a separate incision in the abdominal wall (Mardh *et al.*, 1977b). Serum samples were also collected from an additional 19 women who had no signs of genital infection and who had been laparoscoped.

SERODIAGNOSTIC PROCEDURES

Antibodies to chlamydiae were detected by means of a modified micro-immunofluorescence test using pooled chlamydial antigens (Treharne *et al.*, 1977b). All sera and aspirates from the pouch of Douglas were stored for varying periods of time at -20°C before being tested for different immunoglobulin classes of chlamydial antibodies.

Results

Eighty-eight out of 143 (62%) patients with acute salpingitis had chlamydial IgG antibody levels of 1/64 or greater in their sera (Table 1). The geometric mean titre (GMT) of antibody in all 143 women was 1/51.

The results indicate that there is a correlation between the inflammatory grade of salpingitis, the percentage of women with high levels of IgG chlamydial antibody (that is, $\geq 1/64$), and the GMT of their antibody. Thus a high percentage of women (73%) with severe salpingitis—that is, pelvic peritonitis or abscesses—has significant levels of antibody and also the highest GMT of chlamydial IgG antibody (1/527). In contrast, of the other 19 women with no salpingitis only two showed high levels of antibody. The overall GMT of antibody in this control group was 1/2.

Table 2 Levels of IgM chlamydial antibody in 142 women with acute salpingitis

	No. of patients	With IgM $\geq 1/8$ (%)	GMT of IgM antibody
Grade of salpingitis			
Mild	52	17	1/15
Moderate	61	28	1/24
Severe	29	21	1/58
Totals	142	23	1/25
Controls	19	5	—

The sera of all but one of the 143 women were also examined for the presence of chlamydial IgM antibody (Table 2). In only 33 (23%) out of these 142 women could IgM chlamydial antibody be detected. Although there appeared to be a correlation between the grade of tubal inflammation and the GMT of IgM antibody, no such correlation could be shown with the percentage of women in whom IgM chlamydial antibody was detected.

Fluid aspirated from the pouch of Douglas in 27 women with acute salpingitis had high GMTs of both IgG and IgA chlamydial antibody (Table 3). Once again the GMTs and the percentage of women having high levels of IgG antibody increased with increasing severity of tubal inflammation.

In six women from whom sera and fluid aspirates from the pouch of Douglas were obtained (Table 4) the IgG and IgA chlamydial antibody levels were similar in both sets of specimens except in one patient (case 5), in whom very much higher levels of IgG and IgA chlamydial antibody were detected in the fluid aspirated from the pouch of Douglas.

In 83 out of 88 women in whom IgG chlamydial antibody was demonstrated, the predominant type-specific antibody was of the paratrachoma (D through to K) serotypes. No attempt was made to identify the particular serotype responsible, except in the remaining five patients with salpingitis, in whom the predominant type-specific chlamydial antibody was of the C/J type.

Table 1 Distribution of IgG chlamydial antibody titres in sera from 143 women with acute salpingitis

	No. of patients	Reciprocal IgG chlamydial antibody titres											Geometric mean titre		
		<16	16	32	64	128	256	512	1024	2048	4096	8192	With $\geq 1/64$ (%)	All patients	Patients with IgG antibody
Grade of salpingitis															
Mild	52	14	4	11	12	5	5	1	0	0	0	0	43	1/21	1/63
Moderate	61	11	4	3	10	10	10	5	3	0	3	2	68	1/73	1/189
Severe	30	7	0	1	3	0	7	4	5	2	1	0	73	1/122	1/527
Total	143	32	8	15	25	15	22	10	8	2	4	2	62	1/51	1/160
Controls	19	15	2	0	0	1	1	0	0	0	0	0	10.5	1/2	1/54

Table 3 Distribution of IgG and IgA chlamydial antibody titres in fluid aspirated from the pouch of Douglas in 27 women with salpingitis

	No. of patients	Immuno-globulin	Reciprocal chlamydial antibody titres													With IgG >1/64(%)	GMT of chlamydial antibodies	
			<8	8	16	32	64	128	256	512	1024	2048	4096	8192	16384		IgG	IgA
Grade of salpingitis																		
Mild	12	IgG	1	ND	0	5	1	1	1	1	0	1	0	0	50	1/128	—	
		IgA	6	0	2	1	2	0	1	0	0	0	0	0	—	—	1/45	
Moderate	10	IgG	0	ND	1	1	0	2	1	2	0	1	0	1	80	1/338	—	
		IgA	3	2	0	1	1	1	0	1	1	0	0	0	—	—	1/71	
Severe	5	IgG	1	ND	0	0	0	1	0	0	0	1	1	1	0	80	1/1721	
		IgA	3	0	0	0	1	1	0	0	0	0	0	0	—	—	1/90	
Totals	27	IgG	2	ND	1	6	1	4	2	3	1	2	2	2	1	67	1/285	
Totals	27	IgA	12	2	2	2	4	2	1	1	1	0	0	0	—	—	1/61	

ND Not done

Table 4 Chlamydial antibody titres in matched sera and fluids aspirated from the pouch of Douglas in six women with acute salpingitis

Case no.	IgG reciprocal titre		IgA reciprocal titre	
	Serum	Douglas fluid	Serum	Douglas fluid
1	2048	2048	512	512
2	0	0	ND	0
3	32	32	0	0
4	64	32	ND	0
5	256	4096	0	128
6	128	256	0	8

ND Not done

Discussion

In Sweden, since 1970, the annual number of reported cases of gonorrhoea has decreased by over 30%. During this period, a corresponding decrease in the number of diagnosed cases of PID with gonococcal infection of the cervix has occurred. The total incidence of PID during this same period, however, has increased (Weström and Mårdh, 1975). During the period 1975 to 1977 gonococci could be isolated from the cervix of 10–20% of women with PID in southern Sweden, whereas during the same period *C. trachomatis* was isolated in about 33% of a series of patients with acute salpingitis (Mårdh *et al.*, 1977b).

Laparoscopy offers a means for visual inspection of pelvic organs and thus makes it possible not only to diagnose acute salpingitis accurately but also to collect specimens for culture directly from the fallopian tubes.

A simple serodiagnostic test for the detection of type-specific chlamydial antibody has recently been described (Treharne *et al.*, 1977a). In addition to indicating the predominant type-specific antibody the test can also identify the immunoglobulin class of antibody. The authors showed that women with proved chlamydial cervicitis had a GMT of

IgG antibody of 1/64 or more. Mårdh *et al.* (1977a), using a similar test in two patients with acute salpingitis in whom *C. trachomatis* had been isolated from the fallopian tubes, also showed that high titres of IgG chlamydial antibodies could be detected.

Of 143 women with acute salpingitis described in this present series 62% had IgG chlamydial antibody levels of 1/64 or greater, which we suggest is indicative of an active infection with *C. trachomatis*. Furthermore, only 10.5% of the control group of 19 women showed such levels of antibody, the GMT of all these women being only 1/2. The IgG GMT in the group of women with PID correlated well with the intensity of tubal inflammation, the highest GMT of antibody being found in women with the most severe grade of salpingitis. This pattern of higher GMTs being associated with most severe PID was also found when IgM chlamydial antibody was detected. There was, however, no correlation between the percentage of women having IgM chlamydial antibody and the grade of tubal inflammation as judged by the laparoscopy findings. Overall, only 23% (33 out of 142) of patients had any level of IgM chlamydial antibody. The possible explanation for this finding could be that, as infection of the fallopian tubes is preceded

by cervical infection, it is unlikely that the transiently present IgM antibody would have persisted in the patients with acute salpingitis. There is evidence to suggest that in *C. trachomatis* oculo-genital infections with serotypes generally encountered in genital infections IgM chlamydial antibody persists for about eight to 10 weeks (Treharne *et al.*, unpublished data). Also, the sera from most patients with salpingitis studied had been stored for one to four years before being tested, during which time the relatively labile IgM antibody may have been denatured. Finally, in women developing salpingitis repeated infection with *C. trachomatis* agent may occur, which, if of the same serotype, would apparently result in no recall of the IgM antibody produced during the previous infection (Wang and Grayston, 1971).

The very high GMT of IgG chlamydial antibody (1/285) in fluid aspirated from the pouch of Douglas in two-thirds of the women with PID is strongly suggestive of chlamydial infection. In women with no salpingitis, only two out of nine tested—both of whom had lower genital tract infection—showed any evidence of chlamydial antibody in fluid aspirated from the pouch of Douglas.

Evidence from matched serum and fluid aspirated from the pouch of Douglas in one (Case 5) of the six patients tested suggested that not all the IgG and IgA chlamydial antibody found in fluid aspirates is derived by transudation from the serum but that some may have been produced locally. Indications of local chlamydial antibody have also been shown in cervical secretions from women with cervicitis (Treharne *et al.*, 1978).

Previous studies (Treharne *et al.*, 1977b) showed that mothers of babies with proved ophthalmia neonatorum due to *C. trachomatis* had geometric mean chlamydial antibody titres of 1/122. Dunlop *et al.* (1967) suggested that salpingitis occurs in such women. The patients with salpingitis studied, apart from a group of patients with classical lymphogranuloma venereum and infants with chlamydial pneumonia (Beem and Saxon, 1977), have had the highest levels of antibody of any group of patients with proved *C. trachomatis* infection.

This study, based on serodiagnostic and clinical criteria, indicates that *C. trachomatis* is the probable aetiological agent in as many as 66% of women with acute salpingitis.

The authors are grateful to Professor Barrie R. Jones for his advice and encouragement and to

Mr R. J. Dines and Miss C. Renström for their excellent technical assistance. We are indebted to an anonymous donor and a grant (16X-04509) from the Swedish Medical Research Council for financial support of this collaborative project.

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Ureaplasma urealyticum and *Mycoplasma hominis* in chlamydial and non-chlamydial nongonococcal urethritis

D. TAYLOR-ROBINSON,* R. T. EVANS,* E. D. COUFALIK,*
M. J. PRENTICE,* P. E. MUNDAY,* G. W. CSONKA,† AND J. K. OATES‡

From the *Division of Communicable Diseases, MRC Clinical Research Centre, Harrow, Middlesex, and †St Mary's Hospital and ‡the Westminster Hospital, London

SUMMARY Urethral specimens from 726 patients with nongonococcal urethritis (NGU) were examined for *Chlamydia trachomatis*, *Ureaplasma urealyticum*, and *Mycoplasma hominis*. Chlamydiae were isolated from 35.9% of ureaplasma-positive patients and from 36.5% of ureaplasma-negative patients. Ureaplasmas were isolated from 52.5% of chlamydia-positive patients and from 53.1% of chlamydia-negative patients, an observation which contrasts with that of some workers who have suggested that ureaplasmas are significantly associated with chlamydia-negative NGU. Furthermore, the numbers of ureaplasmas isolated from patients who did or did not harbour chlamydiae were not significantly different nor was there a particular association of ureaplasmas with chlamydia-negative NGU in patients experiencing their first episode of disease. In addition, *M. hominis* was not isolated more frequently from those from whom chlamydiae were or were not isolated. The only significant associations were the isolation of *M. hominis* from patients who were ureaplasma-positive and of ureaplasmas from those who were *M. hominis*-positive. These findings do not necessarily mitigate against ureaplasmas being responsible for some cases of chlamydia-negative NGU.

Introduction

The results of several studies suggest that strains of *Ureaplasma urealyticum* (ureaplasmas) are responsible for some cases of nongonococcal urethritis (NGU). The most compelling evidence for this belief has arisen from human intraurethral inoculation of ureaplasmas (Taylor-Robinson *et al.*, 1977) and from antibiotic trials (Prentice *et al.*, 1976), particularly those in which antibiotics which differentiate between chlamydiae and ureaplasmas have been used (Bowie *et al.*, 1976; Coufalik *et al.*, 1979). It has been suggested by some investigators that ureaplasmas are particularly associated with non-chlamydial NGU. This notion has been based by one group of workers (Wong *et al.*, 1977) on their finding that ureaplasmas were associated only with non-chlamydial urethritis and not with NGU as a whole and by another group (Bowie *et al.*,

1976) on their finding that ureaplasmas were more frequently isolated from patients who were not infected by chlamydiae than from those who were infected. Over the past few years we have examined both clinically and microbiologically more than 700 patients with NGU. *Chlamydia trachomatis*, *Ureaplasma urealyticum*, and *Mycoplasma hominis* organisms were sought, those of the latter two species being quantitatively determined. Because of the quantitative as well as qualitative assessment of the micro-organisms in such a large group of patients, we have been able to evaluate with confidence the inter-relationship among ureaplasmas, *M. hominis*, and chlamydiae in NGU.

Material and methods

PATIENTS

Male patients attending clinics at two hospitals in Central London—namely St Mary's Hospital and the Westminster Hospital—and at two hospitals in or near the Greater London area—namely the Central Middlesex Hospital and Shrodel's Hospital,

Address for reprints: Dr D. Taylor-Robinson, MRC Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ

Received for publication 1 September 1978

Watford—were examined. The diagnosis of nongonococcal urethritis (NGU) was made by passing a bacteriological loop 2 cm into the urethra and Gram staining a smear of the discharge. If gonococci, yeasts, and trichomonads were not seen and 15 or more polymorphonuclear leucocytes were present in one microscope field ($\times 800$ magnification), the patient was adjudged to have NGU and further specimens for microbiological study were taken. Consecutive patients attending each hospital during the period of the study and fulfilling these criteria were included in the study.

COLLECTION OF SPECIMENS AND PROCEDURE

A swab or bacteriological loop was used to inoculate urethral discharge on to GC selective agar medium (Oxoid). These plates were incubated at 36°C in 2% CO₂ in air for 48 hours and examined for the presence of Gram-negative, oxidase-positive diplococci (gonococci). A sterile cottonwool-tipped ENT swab was then inserted about 2 cm into the meatus and expressed into 1.8 ml of mycoplasma liquid transport medium. After a maximum of three hours, these samples were stored at -70°C until tested. They were titrated in urea-containing medium for the detection of ureaplasmas and in arginine-containing medium for *Mycoplasma hominis* (Taylor-Robinson *et al.*, 1971). The presence of these micro-organisms was confirmed by subculture in liquid media and *M. hominis* was identified by the use of specific antiserum in the disc growth-inhibition test. A specimen, for chlamydial culture, was taken by passing a similar swab about 2.3 cm into the urethra. It was expressed into sucrose-phosphate transport medium and stored immediately in liquid nitrogen until inoculated into McCoy cell cultures (Darougar *et al.*, 1971). In some cases, instead of γ -irradiation, the cell monolayers were treated with either 30 μ g/ml of 5-iodo-2'-deoxyuridine for three days before inoculation (Wentworth and Alexander, 1974) or with 1 μ g/ml of cycloheximide immediately after inoculation (Ripa and Mårdh, 1977).

METHODS OF STATISTICAL ANALYSIS

The χ^2 test with Yates's correction was used for comparisons of proportions, and the two-sample Student's *t* test for comparison of means. Results were considered significant if $P \leq 0.05$.

Results

The results of attempts to isolate chlamydiae, ureaplasmas, and *M. hominis* from the urethra of a total of 726 patients with NGU who attended clinics at four hospitals between 1974 and 1978 are presented in Table 1. Chlamydiae were isolated from 36.2% of patients, ureaplasmas from 52.9%, and *M. hominis* from 13.4%.

OCCURRENCE OF UREAPLASMAS OR *M. HOMINIS* IN THE PRESENCE AND ABSENCE OF CHLAMYDIAE

Isolation of ureaplasmas

Ureaplasmas were not isolated significantly more frequently from patients who did not harbour chlamydiae than from those who did, whether or not patients attending each individual clinic or all patients are considered (Table 2). Furthermore, as shown in Figure 1, there was no tendency to recover larger numbers of ureaplasmas from patients who were chlamydia-negative than from those who were chlamydia-positive. Thus, the mean titre among chlamydia-negative patients was $10^{3.78}$ and among chlamydia-positive patients $10^{3.33}$ ($t=1.7$, $P \approx 0.09$).

When 265 patients experiencing their first attack of NGU are considered alone, ureaplasmas were isolated from 61 (57%) of 107 who harboured chlamydiae and from 90 (57%) of 158 who did not, there obviously being no significant difference ($\chi^2 0.014$, $P=0.91$).

Isolation of *M. hominis*

M. hominis organisms were not isolated significantly more frequently from patients who did not harbour chlamydiae than from patients who did, whether or not patients attending each individual clinic or all

Table 1 Isolation of micro-organisms from patients with nongonococcal urethritis

		Patients from whom micro-organisms were isolated						
Hospital clinic	Date seen	Chlamydiae		Ureaplasmas		M. hominis		Total no. of patients
		No.	%	No.	%	No.	%	
Central Middlesex	1974-1975	48	33.3	86	59.7	15	10.4	144
Westminster	1976-1977	51	41.5	55	44.7	13	10.6	123
Shrodell (Watford)	1976-1977	38	29.0	64	48.8	14	10.7	131
Central Middlesex	1976-1977	94	42.9	123	56.2	46	21	219
St Mary's (Paddington)	1978	32	29.3	56	51.4	9	8.3	109
Total		263	36.2	384	52.9	97	13.4	726

Table 2 Isolation of *Ureaplasma urealyticum* from patients who are and are not infected by *Chlamydia trachomatis* or by *Mycoplasma hominis*

Hospital clinic	% of ureaplasma-positive patients among those who are					
	<i>Chlamydia trachomatis</i>			<i>Mycoplasma hominis</i>		
	Positive	Negative	Probability*	Positive	Negative	Probability*
Central Middlesex	50.0	64.5	0.145	100	55.0	0.002
Westminster	51.0	40.3	0.324	84.6	40.0	0.006
Shrodell (Watford)	42.1	51.6	0.425	92.9	43.6	0.001
Central Middlesex	59.6	53.6	0.458	87.0	48.0	<0.0005
St Mary's (Paddington)	50.0	51.9	0.941	100	47.0	0.007
Total	52.5	53.1	0.762	90.7	47.1	<0.0005

*P is significant when <0.05

patients are considered (Table 3). In addition, as shown in Figure 2, the recovery of large numbers of *M. hominis* organisms was not significantly associated with chlamydia-negative patients. Thus, the mean titre among chlamydia-negative patients was $10^{2.03}$ and among chlamydia-positive patients $10^{2.63}$ ($t=1.63$, $P=0.12$).

When patients experiencing a first episode of NGU are considered alone, *M. hominis* organisms were isolated from 16 (15%) of 107 who harboured chlamydiae and from 31 (19.6%) of 158 who did not harbour them, a difference which was also not significant ($\chi^2 0.659$, $P=0.41$).

OCCURRENCE OF CHLAMYDIAE IN THE PRESENCE AND ABSENCE OF UREAPLASMAS OR *M. HOMINIS*

Chlamydiae were not isolated significantly more frequently from patients who did not harbour ureaplasmas than from those who did (Table 4), irrespective of whether or not patients attending each individual clinic or all patients are considered. The same conclusions were drawn about the occurrence of chlamydiae in patients who were or were not infected by *M. hominis* (Table 4).

When patients experiencing NGU for the first time are considered, chlamydiae were isolated from

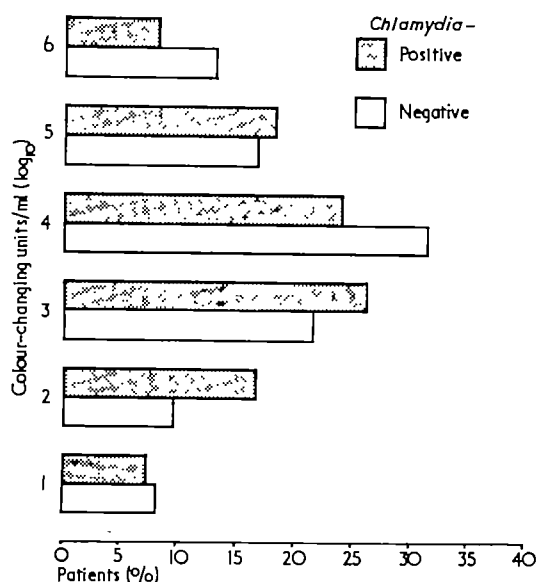


Fig. 1 The quantitative distribution of ureaplasmas (titre expressed as $\log_{10}$ colour-changing units/ml) among chlamydia-positive and chlamydia-negative patients with NGU

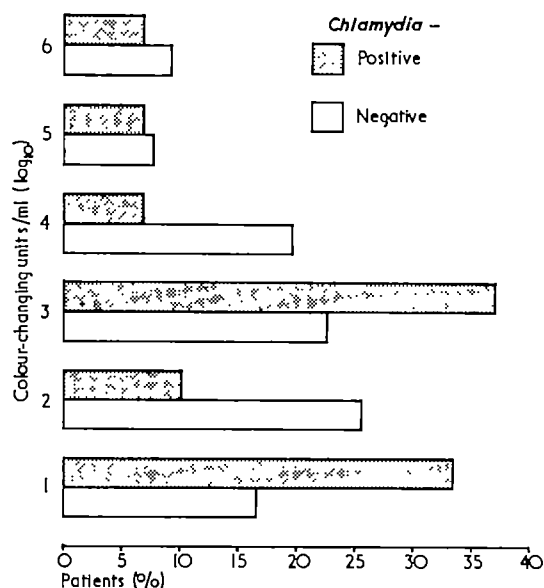


Fig. 2 The quantitative distribution of *M. hominis* organisms (titre expressed as $\log_{10}$ colour-changing units/ml) among chlamydia-positive and chlamydia-negative patients with NGU

Table 3 Isolation of Mycoplasma hominis from patients who are and are not infected by Chlamydia trachomatis or by Ureaplasma urealyticum

Hospital clinic	% of M. hominis-positive patients among those who are					
	Chlamydia trachomatis			Ureaplasma urealyticum		
	Positive	Negative	Probability*	Positive	Negative	Probability*
Central Middlesex	6.25	12.5	0.383	17.4	0	<0.005
Westminster	7.8	12.5	0.594	20.0	2.9	0.006
Shrodel (Watford)	7.9	11.8	0.725	20.3	6.3	<0.001
Central Middlesex	19.1	22.4	0.678	32.5	6.3	<0.005
St Mary's (Paddington)	6.25	9.1	0.932	16.1	0	<0.005
Total	11.4	14.5	0.284	22.9	2.6	<0.0005

*P is significant when <0.05

Table 4 Isolation of Chlamydia trachomatis from patients who are and are not infected by Ureaplasma urealyticum or by Mycoplasma hominis

Hospital clinic	% of chlamydia-positive patients among those who are					
	Ureaplasma urealyticum			Mycoplasma hominis		
	Positive	Negative	Probability*	Positive	Negative	Probability*
Central Middlesex	27.9	41.3	0.144	20.0	34.9	0.383
Westminster	47.3	36.8	0.318	30.8	42.7	0.594
Shrodel (Watford)	25.0	32.8	0.425	21.4	29.9	0.725
Central Middlesex	45.5	39.6	0.458	39.1	43.9	0.932
St Mary's (Paddington)	28.6	30.2	0.94	22.2	30.0	0.932
Total	35.9	36.5	0.762	30.9	37.0	0.284

*P is significant when <0.05

61 (40.4%) of 151 who were ureaplasma-positive and from 46 (40.3%) of 114 who were ureaplasma-negative, obviously an insignificant difference (χ^2 0.014, $P=0.91$). Likewise, in such patients chlamydiae were isolated from 16 (34.4%) of 47 who were *M. hominis*-positive and from 91 (41.7%) of 218 who were *M. hominis*-negative, also an insignificant difference (χ^2 0.659, $P=0.41$).

ASSOCIATION BETWEEN UREAPLASMAS AND *M. HOMINIS*

Ureaplasmas were isolated about twice as frequently from patients who were infected by *M. hominis* than from those who were not infected by this mycoplasma (Table 2), a difference which was significant for patients attending each clinic and for all patients (χ^2 62.56, $P<0.0005$). Conversely, *M. hominis* organisms were isolated more frequently from patients who were harbouring ureaplasmas than from those who were not (Table 3), the difference being significant for patients attending each clinic and for the total number of patients (χ^2 62.56, $P<0.0005$). The difference was also significant (χ^2 22.8, $P<0.0005$) for patients experiencing their first attack of NGU, *M. hominis* being isolated from 27.8% of those infected by ureaplasmas but from only 4.4% of those who were not.

Discussion

Several factors could lead to the isolation of ureaplasmas from patients with NGU and a failure to recover chlamydiae. Since the presence of epithelial cells in a urethral sample is more important for successful chlamydial isolation than for ureaplasma isolation, inadequate swabbing could result in a false predominance of ureaplasma isolates relative to chlamydiae. The same outcome could arise from an inefficient chlamydial isolation technique in the laboratory. We are aware, however, of these problems and consider that our chlamydial isolation technique, both in terms of specimen collection and laboratory procedures, has been satisfactory. Indeed, in studies over several years on a large number of men with NGU, our chlamydial isolation rate has been 36% for unselected patients and about 40% for patients experiencing their first attack. This is in accord with the results recorded by several other groups of workers (Wentworth, 1977). So far as ureaplasmas are concerned, we have found that swab specimens provide an estimate of the numbers of organisms present in the urethra comparable to that obtained by testing urine.

It is difficult to know whether the problems mentioned above could have led others to believe

that ureaplasmas are particularly associated with non-chlamydial NGU. Certainly, from both qualitative and quantitative viewpoints, we can find no basis for the association and we feel confident about this because such a large number of patients has been examined. It is also noteworthy that the data of Holmes *et al.* (1975) do not support the association. A significant association of ureaplasmas with chlamydia-negative NGU might be obscured by patients already possessing ureaplasmas, a situation more likely to occur in persons who have experienced multiple attacks of NGU or who have had multiple sexual partners than in those presenting with a first attack or who had had few sexual partners. We could not assess the influence that the number of sexual partners might have had on ureaplasma isolation, but by examining a large number of patients the unequal distribution among chlamydia-positive and chlamydia-negative groups of those who had, for example, a large number of sexual partners is likely to have been diminished. Certainly, examination of patients experiencing their first attack provided no evidence for the association of ureaplasmas with chlamydia-negative NGU. Furthermore, we have found that *M. hominis* organisms are not isolated more frequently from chlamydia-negative patients and that chlamydiae are not particularly associated with either ureaplasma positive or ureaplasma-negative patients or *M. hominis*-positive or *M. hominis*-negative patients. We have not been able to assess the numbers of chlamydial organisms infecting all the patients, but the qualitative results do not suggest that this would be worthwhile.

Apart from the technical reasons mentioned previously which might lead to false conclusions, one might consider whether it is reasonable for ureaplasmas or *M. hominis* to be particularly associated with chlamydia-negative patients. It is possible to argue that chlamydial damage to cells might provide a situation which is conducive to more prolific growth of ureaplasmas and *M. hominis*, as seen when certain viruses and mycoplasmas are mixed (Reed, 1971). This could lead to an association of ureaplasmas or *M. hominis* particularly with chlamydia-positive urethritis. Again, however, we have not found any evidence that this is so. Indeed, in a situation where there is an opportunity to acquire a variety of micro-organisms one must wonder why patients should be infected by one group of organisms to the exclusion of others. The only significant associations that we have noted are the more frequent isolation of *M. hominis* from men who are infected by ureaplasmas and the more frequent isolation of ureaplasmas from those who harbour *M. hominis*. This is an association which

a number of other investigators, including, for example, Shepard *et al.* (1964) and Kundsins (1976), have remarked on or one which may be deduced from their data. Why this should be is difficult to understand and one can only surmise that the conditions within the genital tract are such that they favour the multiplication and establishment of both these micro-organisms and hence their association.

Our assertion that there is no greater association of ureaplasmas with chlamydia-negative than with chlamydia-positive NGU may raise further doubts in the minds of some workers about the pathogenicity of ureaplasmas. We do not believe, however, that the findings necessarily mitigate against these organisms being responsible for some cases of chlamydia-negative NGU. To propose this would seem as unreasonable as suggesting that chlamydiae have no part to play in nongonococcal disease because they have been isolated by some investigators, such as Richmond *et al.* (1972) and Ridgway and Oriel (1977), as frequently from patients with gonococcal disease as from those with nongonococcal disease. It is equally clear, however, that the present results do not allow us to consider that all non-chlamydial urethritis is ureaplasma. Indeed, the failure to recover ureaplasmas from about 50% of patients with non-chlamydial NGU means that in about 30% of patients with NGU neither of these micro-organisms can be recovered. Unless the present chlamydial and ureaplasma isolation rates are eventually proved to have been underestimated, this suggests that these cases have another aetiology.

We thank the staff of the various hospital clinics for their assistance and Mr D. Altman (CRC Division of Computing and Statistics) for help with the statistical analyses.

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Treatment of nongonococcal urethritis with rifampicin as a means of defining the role of *Ureaplasma urealyticum*

E. D. COUFALIK,* D. TAYLOR-ROBINSON,* AND G. W. CSONKA†

From the *Division of Communicable Diseases, MRC Clinical Research Centre, Harrow, Middlesex, and †the Central Middlesex Hospital, London

SUMMARY The results of a double-blind therapeutic trial on 217 men with nongonococcal urethritis (NGU) show that minocycline was more effective than rifampicin. Before treatment *Chlamydia trachomatis* was isolated from 43% of men, *Ureaplasma urealyticum* from 59%, and *Mycoplasma hominis* from 22%. Chlamydiae and ureaplasmas were isolated less frequently from men with a recent history of NGU. Minocycline was given to 94 patients, and after treatment chlamydiae were isolated from only one of 40 initially chlamydia-positive patients and ureaplasmas from only five of 57 initially ureaplasma-positive patients. Although most patients responded clinically, failure and partial recovery rather than complete recovery were observed more often among those who were infected with ureaplasmas. Rifampicin was given to 123 patients, after which chlamydiae were isolated from only one of 53 initially chlamydia-positive men whereas ureaplasmas, insensitive to the antibiotic *in vitro*, were isolated from 55 of 68 men who had initially positive results. Patients infected with ureaplasmas failed to respond to rifampicin treatment significantly more often than those who were not infected. This was also observed when only patients who had never had NGU or who had not had a recent episode were considered. Furthermore, 24 (44%) of the 55 men whose ureaplasmas persisted failed to recover whereas only one (7.7%) of 13 men whose ureaplasmas disappeared did not respond to treatment. These results suggested that ureaplasmas were a cause of urethritis in some of the men (an estimated 10% at least). In addition, Reiter's disease developed in two men treated with rifampicin from whom only ureaplasmas had been isolated initially. *M. hominis* did not seem to have an important pathogenic role in NGU and there was evidence that ureaplasmas were an unlikely cause of urethritis in some men since the organisms persisted despite complete clinical recovery.

Introduction

There is now considerable evidence to implicate *Chlamydia trachomatis* as one of the causes of nongonococcal urethritis (NGU) (Bowie *et al.*, 1977), although there is still dispute about the way in which chlamydiae cause disease (Richmond and Clarke, 1977). There have been numerous attempts to associate ureaplasmas with NGU and these studies have been discussed recently (Taylor-

Robinson, 1977). Ureaplasmas were previously termed T-strain mycoplasmas, and those of human origin have been ascribed to a new genus and species, *Ureaplasma urealyticum* (Shepard *et al.*, 1974). The results of a placebo-controlled trial of tetracycline in the treatment of NGU (Prentice *et al.*, 1976), human inoculation of ureaplasmas (Taylor-Robinson *et al.*, 1977), and the use of differential antibiotics (Bowie *et al.*, 1976) have provided the most compelling evidence for their involvement in some cases of NGU. In the latter study, urethritis persisted in patients treated with aminocyclitols, which eradicated ureaplasmas but not chlamydiae, and in those treated with sulphafurazole, which

Address for reprints: Dr D. Taylor-Robinson, MRC Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ

Received for publication 17 March 1978

eliminated chlamydiae but not ureaplasmas. The results supported the importance of both chlamydiae and ureaplasmas in the aetiology of NGU.

In the present study of patients with NGU the effect of treatment with a tetracycline (minocycline) was compared with that of rifampicin. Minocycline inhibits the multiplication of both groups of micro-organisms whereas rifampicin inhibits only chlamydiae. Rifampicin has been successfully used in the form of ointment to treat hyperendemic trachoma (Dawson *et al.*, 1975) and paratrachoma (Darougar *et al.*, 1977). If ureaplasmas were responsible for causing urethritis in some patients, we expected that treatment with rifampicin would be less effective than minocycline and that failure to respond would occur mainly in those patients who were infected with ureaplasmas.

Material and methods

PATIENTS

Patients attending the special clinic at the Central Middlesex Hospital who complained of urethral discharge were examined. Urethral discharge was Gram-stained and if 15 or more polymorphonuclear (PMN) leucocytes were present in one microscope field ($\times 800$ – 1000 magnification) and gonococci, yeasts, and trichomonads were not seen, the patient was admitted to the trial. Those patients who had received antibiotics during the preceding six weeks or who had passed urine within the previous four hours were excluded.

ANTIBIOTICS

Rifampicin (Rifadin, Lepetit) is an anti-tuberculous drug. A dose of 600 mg in two tablets was prescribed to be taken 30 minutes before breakfast for six days, giving a total dose of 3.6 g.

Minocycline (Minocin, Lederle) is a long-acting tetracycline and therapeutic blood levels of approximately 2 $\mu\text{g/ml}$ are maintained by 12-hourly oral administration. A loading dose of 200 mg was followed by 100 mg (one tablet) twice a day after meals for six days, giving a total dose of 1.3 g.

EXPERIMENTAL DESIGN

The proposed trial was approved by ethical committees at the Central Middlesex Hospital and the Clinical Research Centre. At the first visit patients were informed that a new treatment was being examined and that it was essential for them to be seen twice more; they were asked to abstain from alcohol and from sexual activity until their dismissal from the clinic. Any patient who admitted to sexual intercourse before that time was withdrawn from the trial. After urethral specimens had been taken

to culture for gonococci, mycoplasmas, and chlamydiae, rifampicin tablets or minocycline tablets (which had a similar appearance) were given to the patients on an approximately alternate basis. This was carried out by a staff nurse-in-charge, who kept a record of the treatment which was not disclosed to either the clinician or the microbiologist until after the end of the trial, when all specimens had been tested microbiologically.

The patients returned after seven to 10 days, having completed the six-day course of the antibiotic. Specimens were again taken for culture of mycoplasmas and chlamydiae. If there was a complaint of persistent discharge or a Gram-stained smear (which was done in all cases) confirmed the presence of any PMN leucocytes, or both, a six-day course of minocycline was instituted.

The third visit was arranged 14 to 21 days after the start of the treatment, when specimens were again taken for culture. When signs and symptoms subsided the patient was discharged from the clinic.

In the text and tables the following terms are used, which are defined thus: complete recovery means no discharge or complaints and no PMN leucocytes in a Gram-stained urethral smear; partial recovery—no discharge but presence of PMN leucocytes in a smear or greatly reduced discharge; failure—as seen originally; and relapse—occurrence of discharge and PMN leucocytes after complete recovery.

COLLECTION OF SPECIMENS AND ISOLATION OF MICRO-ORGANISMS

At the patient's first visit a swab was used to inoculate urethral discharge on to GC selective agar medium (Oxoid). The plates were incubated at 36°C in 2% CO₂ in air for 48 hours and examined for the presence of Gram-negative, oxidase-positive diplococci.

At each of the three visits specimens were collected for mycoplasmal and chlamydial culture. A sterile cottonwool-tipped ENT swab was inserted about 2 cm into the meatus and expressed into 1.8 ml of mycoplasma liquid transport medium. After a maximum of three hours samples were stored at -70°C until tested. They were titrated in urea-containing medium for the detection of ureaplasmas and in arginine-containing medium for *Mycoplasma hominis* (Taylor-Robinson *et al.*, 1968). The presence of micro-organisms was confirmed by subculture in liquid media, and *M. hominis* was identified by the use of specific antiserum in the disc growth inhibition test. All samples were stored again at -70°C .

A specimen for chlamydial culture was always taken second by passing a similar swab 2–3 cm

into the urethra. It was expressed into sucrose-phosphate transport medium and stored immediately in liquid nitrogen until inoculated into McCoy cell cultures (Darougar *et al.*, 1971). In all cases the cells were treated three days before inoculation with 4500 rads of γ -irradiation with a cobalt 60 source.

ANTIBIOTIC SENSITIVITY TESTS

The sensitivity of ureaplasmas to minocycline, erythromycin, and rifampicin was tested by a micro-technique (Taylor-Robinson, 1967), in which each well in the micro-plate contained between 10^3 and 10^4 colour-changing units of the micro-organism. The inhibitory antibiotic concentration was that dilution at which there was complete inhibition of colour change after incubation at 36°C for 18 hours.

Yates's χ^2 test was used for statistical comparisons.

Results

MICRO-ORGANISMS INITIALLY ISOLATED

Of 256 patients seen initially, 39 were excluded because they either had positive culture results for *N. gonorrhoeae* or were not followed up successfully. Thus, there were 217 patients in the trial.

Overall isolation

As shown in Table 1 chlamydiae were isolated from 93 (43%) men, ureaplasmas from 128 (59%), and *M. hominis* from 47 (21.6%). None of these micro-organisms was isolated from 50 (23%) patients.

Isolation related to previous episodes of NGU

Of the 217 patients in the trial, 123 claimed that this was their first episode of urethritis while the remainder had had one or more previous attacks. Chlamydiae were isolated from 44% of patients having a first episode, from 24 (48%) of 50 who had had one or more distant attacks—that is, not within the preceding six months—and from 15 (34%) of 44 of those who had experienced an attack within the previous six months. The latter isolation rate,

however, was not significantly different from the preceding two ($\chi^2=1.98$, $P \approx 0.35$). On the other hand, ureaplasmas were isolated more frequently both from patients without a previous history of NGU (62%) and from those who had had one or more distant attacks (64%) than from those with a recent history (43%), a difference which is significant ($\chi^2=4.91$, $P \approx 0.025$). However, *M. hominis* was isolated with similar frequency from each of the three groups of patients (23%, 22%, and 18%). In view of the above findings it is not surprising that the proportion of patients from whom micro-organisms could not be isolated was significantly greater among those experiencing a recent new attack of NGU (43%) than among those having their first episode (20%) or who had experienced a distant attack (20%) ($\chi^2=9.89$, $P \approx 0.006$).

CLINICAL RESPONSE TO TREATMENT

Minocycline was given to 94 patients and rifampicin to 123 patients (Table 2). Treatment with minocycline was significantly more effective; 68% of patients given this antibiotic recovered completely whereas only 37.4% of patients given rifampicin did so ($\chi^2=24.07$, $P < 0.001$). The proportion of patients who relapsed was about the same whether they were treated with minocycline or rifampicin.

RESPONSE TO TREATMENT WITH MINOCYCLINE IN RELATION TO MICRO-ORGANISMS INITIALLY ISOLATED

Microbiological response

After treatment with minocycline (Table 3), chlamydiae were isolated from only one of 40 initially chlamydia-positive patients, ureaplasmas from five of 57 initially ureaplasma-positive patients, and *M. hominis* from three of 18 persons who had been infected by this mycoplasma.

Clinical response

The one patient who still harboured chlamydiae after minocycline treatment and had failed to

Table 1 *Micro-organisms initially isolated from 217 patients with NGU*

Positive isolation findings	No. of patients	No. of positive findings for each micro-organism		
		Chlamydiae	<i>M. hominis</i>	Ureaplasmas
Chlamydiae alone	34	34		
Chlamydiae and <i>M. hominis</i>	1	1	1	
Chlamydiae, <i>M. hominis</i> , and ureaplasmas	18	18	18	18
Chlamydiae and ureaplasmas	40	40		40
Ureaplasmas alone	46			46
Ureaplasmas and <i>M. hominis</i>	24		24	24
<i>M. hominis</i> alone	4		4	
Total	167 (77%)	93 (43%)	47 (21.6%)	128 (59%)

Table 2 Effect of minocycline or rifampicin on 217 patients with NGU

Clinical response	Treatment			
	Minocycline		Rifampicin	
	No.	%	No.	%
Completely recovered	64	68	46	37.4
Partially recovered	24	25.5	44	35.8
Failure (persistent discharge or PMN or both)	6	6.5	33	26.8
Total	94	100	123	100
Relapsed	8	8.5	11	9

Table 3 Persistence of chlamydiae, ureaplasmas, and *M. hominis* after treatment

Initial isolation*	Proportion of patients† infected by micro-organisms following treatment					
	Minocycline			Rifampicin		
	Chlamydiae	Ureaplasmas	<i>M. hominis</i>	Chlamydiae	Ureaplasmas	<i>M. hominis</i>
Chlamydia-positive ureaplasma-positive (C+U+)	0/28	2/28	0/6	0/30	23/30	9/10
Chlamydia-negative, ureaplasma-positive (C-U+)		3/29	2/9		32/38	10/14
Chlamydia-positive, ureaplasma-negative (C+U-)	1/12			1/23		1/1
<i>M. hominis</i> only			1/3			0/1
Total	1/40	5/57	3/18	1/53	55/68	20/26

*Including *M. hominis*

†The microbiological status of a few patients after treatment was not assessed. The total number of patients in each category is shown in Table 4

respond clinically did so with the disappearance of the organisms after erythromycin was given. Of the five patients who were still infected by ureaplasmas after treatment, one had completely recovered, two had partially recovered, one had failed treatment, and another had relapsed.

The clinical response is further analysed according to the micro-organisms—chlamydiae (C) or ureaplasmas (U)—initially isolated; the patients are placed in four groups according to the positive (+) or negative (−) findings for each organism; C+U+, C−U+, C+U−, and C−U−, as shown in Tables 3 and 4. Few patients failed to respond to treatment and the proportion who did fail was about the same in each group. Because there were so few failures these patients were grouped with those who had a partial recovery for statistical purposes. Although most patients responded, failure and partial recovery rather than complete recovery tended to occur more often among those who were infected with ureaplasmas, the difference being most obvious, but not quite significant, when the C+U+ and C−U+ groups were compared with the C+U− group ($\chi^2=3.18$, $P \approx 0.07$). Other comparisons did not approach a significant value.

The poorer clinical response to minocycline of patients who possessed ureaplasmas appeared to be a feature of those who had experienced a previous recent episode of NGU (Table 5), although the number of patients was small and unsuitable for statistical analysis.

RESPONSE TO TREATMENT WITH RIFAMPICIN IN RELATION TO MICRO-ORGANISMS INITIALLY ISOLATED

Microbiological response

After treatment with rifampicin (Table 3) chlamydiae were isolated from only one of 53 initially chlamydia-positive patients. In contrast, ureaplasmas were isolated from 55 of 68 initially ureaplasma-positive patients, and *M. hominis* from 20 of 26 patients originally infected by this mycoplasma.

Clinical response in relation to chlamydiae

Surprisingly, the one patient from whom chlamydiae were not eradicated had a successful clinical response. In fact, few patients from whom only chlamydiae had been isolated originally failed to respond to treatment (Table 4). Despite failure to isolate the organisms following rifampicin treatment, however, the proportion of patients who completely recovered was smaller than after minocycline treatment.

Clinical response in relation to ureaplasmas

In 13 of the 68 patients ureaplasmas disappeared after treatment. Twelve of these 13 patients completely or partially recovered and only one (7.7%) failed to do so, whereas of the 55 patients whose ureaplasmas persisted 31 completely or partially recovered and 24 (43.6%) failed to recover.

Further analysis of the results (Table 4) shows

Table 4 *Clinical response to treatment in relation to initial microbiological status*

Treatment and clinical response	No. of patients with following initial isolation findings				Total
	C+U+	C-U+	C+U-	C-U-	
With minocycline	28	29	12	25	94
Complete recovery	17	17	11	18	64
Partial recovery	11 (39.3%)	10 (34.5%)	0	4	24
Failed	0	2	1	3	6
Relapsed	1	4	2	1	8
With rifampicin	30	41	23	29	123
Complete recovery	9	16	10	11	46
Partial recovery	9	12	11	12	44
Failed	12 (40%)	13 (31.7%)	2 (8.7%)	6 (20.7%)	33
Relapsed	4	4	1	2	11

Table 5 *Clinical response to treatment in relation to previous disease*

Patients with indicated history of NGU treated with	Initial isolation findings for chlamydiae (C) and ureaplasmas (U)			
	C+U+	C-U+	C+U-	C-U-
Minocycline	Proportion of patients who partially recovered			
None	9/19	8/29	0/6	2/11
Distant	1/17	0/7	0/2	2/4
Recent	1/2	2/2	0/4	0/10
Rifampicin	Proportion of patients who failed to recover			
None	5/14	6/24	1/15	2/14
Distant	5/9	4/9	0/6	3/6
Recent	2/7	3/8	1/2	1/9
	(43%)	(30%)	(5%)	(25%)

that the patients who failed to respond to rifampicin were most frequently those who were originally infected with ureaplasmas. Thus, complete and partial recovery occurred in 46 patients in the combined C+U+ and C-U+ groups and in 44 in the combined C+U- and C-U- groups. Failure to respond, however, occurred in 25 in the C+U+ and C-U+ groups compared with only eight in the C+U- and C-U- groups ($\chi^2=5.04$, $p=0.025$). If the combined C+U+ and C-U+ groups are compared with the C+U- group only (two failures and 21 recoveries in the latter group) the larger number of failures in the ureaplasma-positive groups is again significant ($\chi^2=4.74$, $p\approx 0.03$).

The failure of initially ureaplasma-positive patients to respond to rifampicin was seen about as frequently in those who had had a recent episode of NGU as in the remainder of the patients (Table 5). Placing these latter patients, from whom micro-organisms were isolated most frequently, into groups as previously—that is, C+U+, C-U+, C+U-, C-U—showed that treatment failures occurred more often in the groups containing ureaplasmas (Table 5) ($\chi^2=4.34$, $p\approx 0.04$), as noted previously for the patients as a whole (Table 4).

A closer examination of the data on the 31 patients who were initially infected by ureaplasmas and who completely or partially recovered showed that the organisms were unlikely to be the cause of

disease in at least nine of them. The latter patients completely recovered and remained well for up to three weeks, even though at the time of the second and third visits ureaplasmas were isolated in numbers similar to those observed initially.

Clinical response in relation to M. hominis

As shown in Table 1, *M. hominis* was isolated predominantly from patients who were infected with ureaplasmas. It was possible that the failure of ureaplasma-positive patients to respond to rifampicin was due to their concurrent infection with *M. hominis*. To assess this, the clinical response of patients infected with *M. hominis* was compared with the response of those who were not infected (Table 6). The proportion of patients who failed to respond to treatment was about the same in the U+*M. hominis*+ group as in the ureaplasma-only group. In contrast, the proportion who failed to respond in the U+C+*M. hominis*+ group was greater than in the U+C+ only group. Further analysis, however, indicated that failure to respond in the former group might be attributable to *M. hominis* in only one of the five patients.

Relationship of disease severity to micro-organisms isolated and to clinical outcome

To determine whether differences in the severity of disease were associated with particular micro-organisms the symptoms and signs were correlated

Table 6 Clinical response to rifampicin of patients who were infected or not infected by *M. hominis*

Group treated	No. of patients	Complete recovery	Partial recovery	Failed		
				No.	%	Relapse
U+ <i>M. hominis</i> +	15	5	5	5	33	3
Ureaplasmas only	26	12	7	7	27	1
U+C+ <i>M. hominis</i> +	10	3	2	5	50	1
U+C+ only	20	6	9	5	25	3

with the organisms initially isolated in two groups of patients, namely those from whom chlamydiae but not ureaplasmas were isolated and those from whom ureaplasmas but not chlamydiae were isolated. The amount of discharge was based on a +, ++, +++ score denoting minimal, moderate, and severe, and the total score for all patients within a group was divided by the number of patients to obtain an average discharge score. The number of polymorphonuclear (PMN) leucocytes observed on a Gram-stained smear was likewise summarised for all patients and divided by the number of patients to obtain an average PMN leucocyte score. For patients who were chlamydia-positive only, the average discharge score was 1.94 and the average PMN leucocyte score 19.7. For patients who were ureaplasma-positive only, the average discharge score was 2.16 and the average PMN leucocyte score 20.3. There was, therefore, no evidence that the presence of one or other of these micro-organisms was associated with more severe disease.

Data were analysed on the group of patients in which most treatment failures occurred—namely those patients treated with rifampicin who were initially chlamydia-negative but ureaplasma-positive—to determine whether severe disease resulted in failure to recover clinically. There was no evidence for this because the average discharge score was 2.0 and the average PMN leucocyte score was 19.2 for those who failed to respond, and 2.0 and 18.4 respectively for those who recovered.

Antibiotic sensitivity tests

Chlamydiae persisted in one patient after minocycline treatment and in one patient after rifampicin treatment (Table 3), but unfortunately the organisms were not available for antibiotic sensitivity tests. Ureaplasmas persisted in five patients after minocycline treatment (Table 3) but were sensitive to 0.01 µg/ml of the antibiotic. Ureaplasmas were not recovered from 13 patients following rifampicin treatment, but apparently this was not due to the effect of the antibiotic because the organisms initially isolated from these patients were resistant to 80 µg/ml of rifampicin.

Development of Reiter's disease

Ureaplasmas but not chlamydiae were isolated from the urethral discharge of two patients. Both patients were treated with rifampicin and the ureaplasmas and discharge persisted but these had disappeared two weeks after the initial visit to the clinic following a course of minocycline. The first signs of Reiter's disease were observed one week after the initial visit in the case of the first patient and two weeks after in the case of the second.

Discussion

In this study we isolated chlamydiae and *M. hominis* more frequently than we had done previously (Prentice *et al.*, 1976), and the rate of isolation of both chlamydiae and ureaplasmas was comparable with that observed by other workers (Wentworth, 1977; Taylor-Robinson, 1976). Thus, the recovery of micro-organisms was sufficiently satisfactory to enable us to correlate the microbiological results with the outcome of treatment. As noted by others (Alani *et al.*, 1977) and previously by ourselves (Prentice *et al.*, 1976), a past history of NGU was associated with a lower isolation rate. This occurred, however, only if there had been a previous episode within the six-month period immediately preceding the current infection.

Bowie *et al.* (1976), in part of their study, used aminocyclitols which eradicated ureaplasmas but not chlamydiae. Since there is now considerable evidence, as mentioned previously, that chlamydiae are pathogens in the male genital tract, we considered that it would be unwise to withhold treatment from chlamydia-positive patients. We, therefore, did not use a drug which was ineffective against chlamydiae but confined the study to a comparison of rifampicin, which inhibits chlamydiae but not ureaplasmas, with minocycline, which inhibits the multiplication of both micro-organisms. Chlamydiae could not be recovered from the urethra following rifampicin treatment, and indeed there was no evidence that rifampicin-resistant strains emerged as the findings of other workers (Keshishyan *et al.*, 1973) suggested might happen. Despite apparent

eradication rifampicin was less effective in treating chlamydia-positive patients than minocycline, which may have been due to the greater bactericidal activity of the latter drug (Treharne *et al.*, 1977). It was interesting to note that treatment with minocycline was not so successful clinically when ureaplasmas, with or without chlamydiae, were present. This in itself may be evidence for a differential antibiotic effect, the antibiotic destroying chlamydiae but having a more static than cidal effect on ureaplasmas. Failure to respond to therapy on account of the presence of minocycline-resistant ureaplasmas, however, was not encountered, although such strains have been found previously (Evans and Taylor-Robinson, 1978).

Most patients who failed to respond to treatment had been given rifampicin, and failure occurred significantly more frequently in those patients who were infected with ureaplasmas than in those who were not. This observation is in keeping with that of Bowie *et al.* (1976), who found that sulphafurazole—which eliminated chlamydiae but not ureaplasmas—was less successful when patients were infected by the latter micro-organisms than when they were not. The results of both studies suggest that ureaplasmas are a cause of some cases of NGU, although, as indicated below, there are problems in evaluating the data.

The group of 29 patients who were treated with rifampicin and from whom chlamydiae and ureaplasmas were not isolated initially (C-U-) is virtually a micro-organism-negative group comprising only one *M. hominis*-positive patient. Whether the C-U- group of patients is one with which the other groups may be validly compared is an aspect which influences the interpretation of the results. Thus, while a greater proportion of patients who possessed ureaplasmas failed to respond to treatment than those who did not (C+U- and C-U- groups combined or C+U-), this was not so if the groups possessing ureaplasmas were compared with the C-U- group, an aspect not mentioned by Bowie *et al.* (1976). The question arises of whether the only difference between the C-U+ group and the C-U- group of patients is the absence of ureaplasmas or whether the latter group of patients is infected by different micro-organisms entirely and is therefore unacceptable for comparative purposes. It seems rather likely that the C-U- group does not comprise undetectable chlamydiae—preliminary serological studies providing further evidence for their absence (B. Thomas, personal communication)—although the existence of a few, as yet, undetectable ureaplasmas has not been eliminated. The available evidence suggests, therefore, that the group is

somewhat different from the others and should not be used for comparative purposes. This belief is reinforced by the fact that patients in the C+U- group did not respond significantly better to rifampicin than those in the C-U- group, an unlikely result if the only difference between the patients in the two groups was the possession of chlamydiae by the former. The fact that a substantial proportion of the patients within the C-U- group responded to antibiotics suggests that they were infected with antibiotic-sensitive organisms, and there is clearly a need to establish what these were.

In recent times, *M. hominis* has not been seriously considered as a potential pathogen in the male urogenital tract. Nevertheless, this mycoplasma cannot be ignored in the present study because it is also insensitive to rifampicin, so that treatment failures attributed to ureaplasmas could be due to *M. hominis* in those cases where it co-existed. The results suggest the *M. hominis* is, at the most, rarely pathogenic. The study, however, was not designed to evaluate the role of this mycoplasma; this might best be done by treating the C-U+*M. hominis*+ group of patients with lincomycin, which inhibits *M. hominis* but not ureaplasmas, and with erythromycin, which has the reverse effect.

As expected, ureaplasmas which initially infected patients usually persisted after rifampicin treatment. In some cases, however, the organisms could not be reisolated and their disappearance was not due to their sensitivity to the antibiotic. This is the first evidence of spontaneous clearance of ureaplasmas from adult male patients and contrasts with the results of previous studies where persistence for at least a year was noted in the absence of antibiotic therapy (Holmes *et al.*, 1974; MacLeod *et al.*, 1976). Patients in whom ureaplasmas disappeared recovered more frequently than those in whom the organisms persisted. This observation might be used as evidence for a causative role of ureaplasmas but it is not possible to know whether the patients improved because the organisms disappeared or whether the organisms were not recoverable because the disease subsided spontaneously, isolation being less easily achieved in the absence of discharge. The latter possibility seems less likely, however, because there were several examples of patients who made a complete clinical recovery and yet ureaplasmas persisted in numbers similar to those observed initially. In such cases it seems unlikely that the ureaplasmas were responsible for the disease. What proportion of patients might have urethritis accountable for by ureaplasma infection is therefore an obvious question. In the C-U+ group treated with rifampicin, 13 of 41 patients failed to respond. Thus, if all 70 patients in the C-U+ group—that

is, those treated with rifampicin and minocycline—had in fact been treated with rifampicin alone, it can be calculated that 22 of them would have failed to respond as a result of possessing ureaplasmas. It would seem reasonable to consider, therefore, that a minimum of 10% of patients (that is, 22/217) had a urethritis due to ureaplasmas. In patients infected by chlamydiae the ureaplasmas may, of course, contribute to the disease. It is interesting that in both of the patients who developed Reiter's disease ureaplasmas but not chlamydiae were isolated from the urethral discharge of each of them. Whether inadequate treatment with rifampicin contributed to the development of the disease is a moot point. The intriguing question of whether ureaplasmas play a significant role in Reiter's disease can only be answered by undertaking a planned prospective study in which patients with NGU are assessed microbiologically before they develop Reiter's disease (Taylor-Robinson, 1979).

We thank the staff of the clinic at the Central Middlesex Hospital for their help in this study, Mr R. T. Evans and Mrs Mary Osborn for some technical assistance, and Mr D. Altman for help with the statistical analyses.

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Herpesvirus hominis antibodies among children and young adults in Ibadan

A. O. SOGBETUN, D. MONTEFIORE, AND C. N. ANONG

From the Department of Medical Microbiology, University College Hospital, Ibadan, Nigeria

SUMMARY A total of 422 sera collected from children and young adults living in Ibadan, Nigeria, has been examined for the presence of antibodies to type 1 and type 2 herpesvirus hominis. Type 1 antibodies were rapidly acquired from the age of 1 year onwards, reflecting the relatively poor living standards and the overcrowded accommodation among the population studied. Type 2 antibodies were acquired between the ages of 3 and 5 years. It is suggested that non-venereal spread of the virus must be responsible; prolonged survival of the virus on fomites, owing to the high environmental humidity, possibly accounts for this.

Introduction

Several of the herpesviruses which infect animals possess oncogenic properties, and a causal association is suspected between infection with herpesvirus hominis type 2 (HVH-2) and later development of carcinoma of the uterine cervix (Rapp, 1973), which is one of the commonest malignant conditions seen in women in Ibadan (Attah and Hendrickse, 1977). Genital herpes, almost exclusively due to HVH-2 (Amstey, 1973), is being diagnosed with increasing frequency (WHO, 1974), although it is not clear whether there is a true increase in the incidence of infection or whether the increased frequency in diagnosis results from better diagnostic methods. However, much of the evidence which has brought HVH-2 under suspicion as a possible cause of cervical carcinoma derives from sero-epidemiological studies, and there is, therefore, considerable interest in the incidence of infection with this virus in different parts of the world.

There is little information available concerning genital herpetic infection in Nigeria, although preliminary serological studies by Adelusi *et al.* (1975, 1977) suggest that approximately 13% of healthy young women may have experienced previous infection with the virus. Genital herpes infection is not often seen in the acute stage in hospital practice, but this may be due to the fact that where medical facilities are relatively scarce only patients with the most extensive and painful

lesions attend. Those patients with milder lesions are likely to treat themselves or seek advice from native practitioners.

Patients acquire herpesvirus hominis infection as a result of close personal contact (Nahmias and Roizman, 1973). This contact is generally non-sexual in the case of the type 1 virus and sexual in the case of the type 2 virus. The evidence for considering HVH-2 infection as a sexually transmitted disease is based on the finding that antibodies to the virus are virtually absent from children below the age of puberty (Nahmias *et al.*, 1970) and among celibate communities such as nuns (Nahmias and Roizman, 1973), and also that antibodies occur most frequently among the sexually promiscuous (Duenas *et al.*, 1972). In addition, type 2 virus is isolated from approximately 90% of genital lesions, while type 1 virus is isolated from a similar proportion of 'above-the-waist' infections (Dowdle *et al.*, 1967).

Antibodies to type 1 virus start to increase in frequency from about 6 months of age, once maternally acquired immunoglobulins have disappeared. The rate of acquisition, and the final proportion of sera found to contain type 1 antibodies, varies with the population studied. Both variables tend to be higher among populations of low socioeconomic status (Smith *et al.*, 1967), probably reflecting greater opportunities for spread of the virus where living conditions are crowded.

These data, however, derive from populations studied in the developed countries, and the distribution and incidence of antibodies to the two types of virus may not necessarily follow the same pattern

Address for reprints: Dr A. O. Sogbetun, Department of Medical Microbiology, University College Hospital, Ibadan, Nigeria

Received for publication 5 July 1978

among people living under the very different conditions of a developing tropical country. Under the latter conditions type 2 virus could probably spread more readily by non-venereal routes (Poste *et al.*, 1972; Roome *et al.*, 1975), and this might be reflected by an increased incidence of type 2 antibody among the younger age groups.

The present paper reports the incidence of antibodies to herpesvirus hominis types 1 and 2 among children and young adults living in Ibadan, a large city in the humid tropics. The population samples in the younger age groups included children attending clinics at the University College Hospital, Ibadan, for conditions unrelated to herpetic infection while those in the older age groups included healthy school pupils.

Material and methods

SERA TESTED

Fifty-two samples of sera were collected from children in the age group 0-3 months; of these 29 were cord blood samples obtained in the labour wards of the hospital and 23 were capillary blood samples from children attending clinics for conditions unrelated to herpesvirus infection. In the latter instances, approximately 0.5 ml of blood was collected by heel or finger prick. Sera were separated and stored at -20°C before being tested.

In the older age groups venous blood, or in younger children capillary blood, samples were similarly collected and the sera separated and stored at -20°C before being tested. In the older age groups samples were obtained from healthy school pupils.

In all cases, the children were broadly representative of the socioeconomic level of the majority of the Ibadan population. Serum samples were not collected from children of parents in the higher socioeconomic groups.

NEUTRALISATION TESTS

Serum samples were examined by the micro-neutralisation test in disposable flat-bottomed plastic tissue culture plates. Serum titrations were carried out against known type 1 and type 2 virus strains. Control sera from rabbits initially immunised against the respective virus were included in each batch of tests. Both the type 1 and the type 2 antisera were first tested 78 times against each type of virus, the pN values determined, and the ratio pN type 2:pN type 1 calculated. This ratio, multiplied by 100, was termed the NpN value.

The method of testing the sera and calculating the NpN values was based on that of Pauls and Dowdle

(1967) with the modifications described previously by Roome *et al.* (1975). The initial serum dilution in each case was 1/10, as higher serum concentrations tended to be toxic to the Vero cells used. Results for sera not showing neutralisation at a 1/10 dilution were regarded as negative.

The mean NpN value for each serum sample was obtained together with the standard deviation for each set of values. For the type 1 antiserum the mean NpN value was 78.5 while for the type 2 antiserum the mean NpN value was 113.2, with standard deviations of 6.5 and 15.2 respectively. When the results of test sera were interpreted, those which gave NpN values identical with those of the control sera (standard deviation ± 1) were regarded as containing only a single antibody of the appropriate type. Those sera with NpN values which fell between the control values (standard deviation ± 1) were considered to contain both types of antibody.

Results

The results for the total of 422 children and young adults examined are shown in the Table. The numbers of subjects in each age group are indicated, together with the number of serum samples containing either type 1 antibody, type 2 antibody, or no detectable antibody. Where a serum sample contained both types of antibody it has been included in both categories. Of the 422 subjects 210 were female and 212 were male; the findings did not differ for the two sexes in any age group and so the results for both sexes have been combined.

In the 0-3 month age group, 86% of sera showed the presence of type 1 antibodies. This percentage fell to 6.9% in the 4-11 month age group, reflecting waning maternal antibody, but then rose rapidly from 1 year of age onwards to reach a peak of between 70% and 83% in the teenage period.

Type 2 antibodies were present in approximately 6% of sera in the youngest age group, with the incidence falling to only 1.6% by 3 years of age. Between 3 and 5 years, however, there was a rapid acquisition of type 2 antibodies, with the incidence rising to approximately 11%; only a slight further increase occurred in the older age groups.

Discussion

There have been relatively few published reports on the age-related acquisition of antibodies to herpesvirus hominis, and in the earlier studies the methods used did not distinguish between antibodies to the two different types of virus. Thus Smith *et al.* (1967) noted that in the Edinburgh population antibodies

Table Incidence of antibodies to herpesvirus hominis in sera from children and young adults

Age group	No. of sera tested	Positive herpesvirus hominis antibodies					
		Type 1*		Type 2*		No. antibodies	
		No.	%	No.	%	No.	%
0-3 months	52	45	86.5	3	5.8	6	11.5
4-11 months	29	2	6.9	1	3.5	26	89.7
1-2 years	64	17	26.6	1	1.6	46	71.9
3-5 years	64	36	56.3	7	10.9	22	34.4
6-10 years	49	37	75.5	7	14.3	7	14.3
11-15 years	82	68	82.9	10	12.2	10	12.2
16-20 years	82	58	70.7	10	12.2	16	19.5

*Sera containing both types of antibody are included in both columns

to herpesvirus hominis were present in 100% of samples from the 0-3 month age group, with the incidence falling to a low point of 19% between 6 and 12 months and thereafter rising gradually to around 70% by the age of 20 years. These workers particularly noted the effect of socioeconomic status on the incidence of herpesvirus hominis antibodies, with the proportion of sera giving positive results decreasing as living standards improved. More recently Terzin and Masic (1976), in Yugoslavia, noted a similar pattern of antibody response to type 1 infection, although among their population infection seemed to be acquired at a much earlier age than in Edinburgh. Our findings in Ibadan are similar to those in Yugoslavia and may be taken to reflect early acquisition of herpesvirus infection as a result of relatively unsatisfactory living conditions and consequent overcrowding.

Nahmias *et al.* (1970) studied the acquisition of antibodies to the two types of virus in an American population. Type 1 infections were acquired less rapidly than in Yugoslavia or in Ibadan—presumably because of a higher standard of living and better accommodation. Type 2 antibodies were detectable, once maternally acquired immunoglobulins had waned, only in children over 14 years old, a finding that has been much quoted in support of the view that type 2 infection is mostly spread by sexual contact. The findings of Duenas *et al.* (1972) also support this view, since the incidence of type 2 antibodies increased with age and professional experience among the group of prostitutes examined by these workers.

Infection with type 2 herpesvirus hominis is not unknown among young children (Nahmias *et al.*, 1968), although it is apparently very uncommon. In Ibadan, however, our serological evidence indicates that infection with this virus occurs at a young age, with approximately 11% of sera from children aged between 3 and 5 years containing antibody. These findings resemble the observations of Osoba and Alausa (1974), who found that

gonococcal vulvovaginitis in young girls was relatively frequent in Ibadan and that sexual assault did not appear to be responsible. They suggested that the gonococcus was transmitted from older relatives to young children, not only by close personal contact but also by shared towels and bed clothes. This suggestion received support from the findings of Alausa *et al.* (1977) that while *Neisseria gonorrhoeae* in simulated exudates was indeed rapidly destroyed by drying (Wilson and Miles, 1975) this process was delayed, and viable gonococci persisted for at least several hours, when the samples were deposited on cloth and exposed to the humid air during the rainy season in Nigeria.

Thus the common habit among the local population of young children sleeping in the same bed as older relatives, as well as sometimes using the same towels and articles of clothing, could well lead to transmission of infection to the young child without the necessity of close or immediate bodily contact.

The possibility of non-venereal spread of herpesvirus hominis type 2 infection has been previously noted by Poste *et al.* (1972) and Roome *et al.* (1975), and in Ibadan it seems possible that such spread may be of particular importance. The same situation may exist with regards to the survival of herpesvirus in the tropics as has been previously shown in the case of the gonococcus, and could similarly lead to an increased incidence of HVH-2 infection among young children.

We would like to thank Dr A. P. C. H. Roome for kindly supplying the type strains of virus.

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Necrotising balanitis due to a generalised primary infection with herpes simplex virus type 2

J. F. PEUTHERER, ISABEL W. SMITH, AND D. H. H. ROBERTSON

From the Departments of Bacteriology and Venereology, Edinburgh University Medical School, and the Royal Infirmary, Edinburgh

SUMMARY Virological studies have proved herpes simplex virus type 2 to be the cause of the severe primary infection in a 37-year-old man with necrotising balanitis. Symptoms of urethritis preceded the appearance of a severe local lesion; dissemination of infection occurred and lesions developed on the skin of the scalp, neck, trunk, buttocks, arms, legs, and feet. No evidence of involvement of the central nervous system was found, and the penile and skin lesions improved rapidly after the application of 5% idoxuridine in dimethylsulphoxide. Recurrences occurred at various skin sites at six to seven months after the primary infection.

Introduction

Almost 90% of genital infections in men are caused by herpes simplex virus type 2 (HSV-2) strains; in one-third of these patients the HSV-2 infection occurs in the absence of antibody to either HSV-1 or HSV-2 (Smith *et al.*, 1976). We describe an unusually severe primary HSV-2 infection in a 37-year-old man.

Case report

HISTORY

A 37-year-old Caucasian man was first seen on 15 January 1976 complaining of pain in the penis and inguinal region, dysuria, and a slight urethral discharge for two days, which had developed about five days after sexual intercourse with an unknown consort. On examination he was noted to have a blood-stained urethral discharge and injection of the meatus. Urine analysis showed protein and glucose. On the basis of the microscopical appearance in the urethral discharge of leucocytes but no organisms a provisional diagnosis of non-specific urethritis was made; the patient was given oxytetracycline 250 mg six-hourly for five days and asked to return four days later on 19 January 1976.

On his return the patient said that he had become less well and that he had attended his own general

practitioner complaining of dyspnoea and dizziness. He also said that he had been confused during this period. He was admitted to the ward. On examination there was a shield-shaped blackened area of skin surrounding the urethral meatus and flattened pustules which were irregular in outline and peripheral to this necrotic area (Fig. 1). The glans, prepuce, and shaft of the penis were oedematous; the shaft was hot and reddened with some induration along the urethra. Dome-shaped pustules (Fig. 2) varying in size from 1 mm to about 7 mm were widespread over the skin surface and distributed approximately as follows: scalp, one; neck, one; chin, one; trunk, six; buttocks, about 40; scrotum, two; fingers, (right) one and (left) one; shoulder, (right) one; upper arm, nil; thigh and leg, (right) 17 and (left) four; and foot, (right) two and (left) four. The patient was febrile (38.5°C) and looked unwell. Apart from drowsiness and slight confusion at times no abnormality was noted in the nervous system. A technetium brain scan showed normal uptake without any areas of increased activity to indicate cerebral inflammation or abscess formation. An echo encephalogram showed no shift of midline structures. No abnormality was found in the electroencephalogram.

LABORATORY RESULTS

The initial erythrocyte sedimentation rate was 74 mm in the first hour. The white cell count was $7.8 \times 10^9/l$ ($7.8 \times 10^3/mm^3$); 69% neutrophils, 25% lymphocytes, 5% monocytes, and 1% eosinophils.

Address for reprints: Dr J. W. Smith, Department of Bacteriology, Edinburgh University Medical School, Teviot Place, Edinburgh EH8 9AG

Received for publication 15 August 1978



Fig. 1 Penile lesion

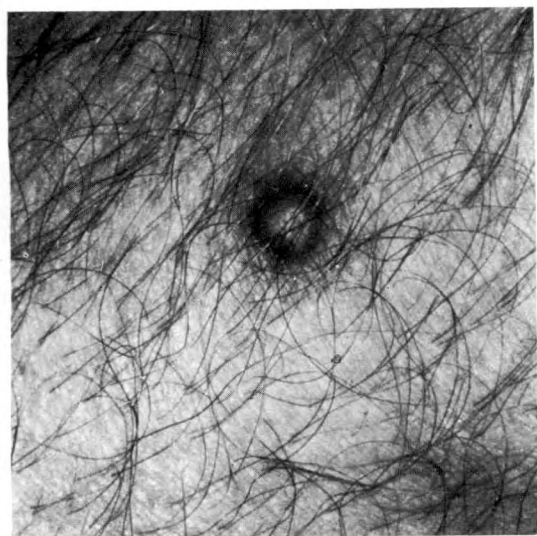


Fig. 2 Dome shaped pustule

Total serum protein, albumin, electrophoretic pattern, and immunoglobulins (IgG, IgM, IgA, and IgE) were within normal limits.

Subsequently he was shown to have a normal population of T and B lymphocytes, which gave a

good transformation response in culture when stimulated by phytohaemagglutinin (PHA) and concanavalin A (con A). He had positive skin test reactions to purified protein derivative (PPD), mumps, *Candida*, SKSD, and *Trichophyton* antigens.

Bacteriology

Neisseria gonorrhoeae was not isolated and the Venereal Disease Research Laboratory and *Treponema pallidum* haemagglutination tests gave negative results on the four occasions they were performed over eight months starting on the day of admission.

Virus isolation

Herpes simplex virus was isolated in human embryo lung cell cultures from swabs of the glans and of a pustule on the thigh on 19 January; from swabs of pustules on the foot, scapular region, and fourth finger (right hand) on 23 January; and from swabs of a neck pustule on 26 January.

Virus isolation was unsuccessful from a urethral scrape on 9 February and from a scrape of the genital lesion and of the fluid and roof of a vesicle on the foot on 24 February.

The isolates were confirmed as type 2 strains by the biological typing features cited by Smith *et al.* (1973).

Serology

Serum specimens were collected during the acute illness and up to almost 13 months later. The sera were tested in chessboard titrations against type 1 and type 2 herpes simplex virus antigens in complement-fixation and in neutralisation tests (Smith *et al.*, 1967). The serological conversion measured by the neutralisation test and the significant response shown with both types of complement-fixing antigens established that the patient had a primary herpetic infection (Table). The relative titres of complement-fixing and neutralising antibodies are in agreement with the unpublished observations of Smith and Peutherer.

Analysis of the chessboard complement-fixing titration indicated that the response was greater to the type 2 antigen than to the type 1 antigen. This result is in agreement with the virus type isolated from the patient. Maximal complement-fixing titres of 512 were recorded during the acute illness.

Each serum at a 1/10 dilution was tested by an indirect immunofluorescence test for IgM antibody to HSV-1 and HSV-2; sera with positive results were titrated. Sera gave negative results when tested by a latex agglutination test for rheumatoid factor, and IgM antibody was detected in the lower fraction from a sucrose density gradient (10–40%) spun for 16 hours at 100 000 $\times g$.

Table Results of serological tests

Date of collection	Reciprocal titre to HSV-2			Reciprocal titre to HSV-1		
	N	CF	IgM	N	CF	IgM
19 January 1976	<8	128	320	<8	128	160
26 January 1976	64	512	ND	128	512	ND
9 February 1976	256	512	160	128	512	80
25 February 1976	256	512	160	128	256	40
19 August 1976	128	256	<10	128	256	<10
3 November 1976	>256	256	<10	128	256	<10
9 December 1976	>256	128	<10	256	128	<10
31 January 1977	>256	256	<10	128	128	<10
7 February 1977	>256	128	<10	128	64	<10

N = Neutralising antibody
 CF = Complement-fixing antibody
 ND = Not done

Specific IgM antibody was detected during the acute stages of the primary infection and for at least four weeks thereafter (Table).

Histology and electron microscopical examination

A biopsy specimen of a neck pustule (taken on 26 January) was fixed, stained, and examined in both the light and electron microscopes. Giant cells were seen by light microscopy.

Ultrathin sections were examined in the electron microscope; large numbers of virions were present but no intracellular filaments or microtubules could be detected.

TREATMENT ON ADMISSION

Cellulitis of the penis and a septicaemia or viraemia were suspected when the patient was admitted to the ward. The fever settled gradually while the patient was on crystalline benzyl penicillin 1 mega unit four-hourly, but his temperature rose to 38.5°C after the treatment was changed to ampicillin 500 mg orally every six hours. Surgical debridement of the penile lesion under anaesthesia had been arranged, but once herpes simplex virus type 2 (HSV-2) had been isolated the penile lesion and the bases of all the opened and evacuated pustules were painted with a 5% solution of idoxuridine in dimethylsulphoxide. This procedure was repeated three times a day for five days and was followed by very rapid improvement. All pustules began to heal and the penile lesion improved with separation of the necrotic slough. Healing continued gradually and the patient was discharged on 26 February 1976.

PROGRESS

At follow-up examination on 3 March meatal stenosis was present and this was treated by dilatation. The patient remained well until 9 December,

when he developed a cluster of vesicular lesions on the right heel; HSV-2 was isolated from the lesions.

The patient then presented at the end of January with lesions on the left foot and the neck at the site of the primary lesions, on the right side of the abdomen, and on the left ring finger. HSV-2 was recovered from these lesions.

Complement-fixation titres remained at 256 during the period of recurrences; IgM specific for HSV-2 was not detected during this time (Table). The patient has remained symptom free to date.

Discussion

This patient had an unusually severe infection with HSV-2. The local genital lesion was extensive and necrotic and was comparable in severity to the necrotic cervicitis described by Willcox (1968). Initially, a diagnosis of non-specific urethritis was made. The progression from urethritis to both a severe local lesion and disseminated skin involvement occurred over a period of four days. HSV-2 was isolated from the pustules; this contrasts with the skin lesions associated with gonococcal septicaemia, in which an allergic vasculitis is believed to be important in their pathogenesis (Scherer and Braun-Falco, 1976).

Dissemination of virus from a genital site of infection would be expected to occur only in patients who had not been infected with either virus type. The serological evidence established that the patient had a primary type 2 infection. It is of interest that the reciprocal complement-fixing titres were still elevated (256) one year after the onset. The recurrent episodes within one year of the primary infection are comparable to the pattern of those reported by Yoshino *et al.* (1962) after primary oral infection with HSV-1. Similarly, the detection of specific IgM during the primary

infection, but not during the recurrent episodes, is in agreement with previous results (Kurtz, 1974). We have detected IgM antibody, however, in the serum of other patients during recurrences or reinfections with the homologous and heterologous virus types (Smith *et al.*, unpublished data).

Disseminated infection with herpes simplex virus type 2 has been described in a young adult (Juel-Jensen and MacCallum, 1972). The fatal case described by Sutton *et al.* (1974) occurred in a 20-year-old woman with dysplasia of the thymus. In our case, an assessment of the patient's humoral and cell-mediated immune responses to various stimuli at the time of the recurrences showed no abnormality. It was not possible to test his response to HSV-1 and HSV-2 antigens. An association between type 2 genital infection, viraemia, and involvement of the central nervous system has been claimed (Craig and Nahmias, 1973), and Skoldenberg and Jeansson (1973) have reported the isolation of type 2 strains of virus from the cerebrospinal fluid of seven patients with acute aseptic meningitis. There was no clear evidence of meningitis or encephalitis in our patient, although he complained of dizziness and confusion during the interval between his first and second visits to the clinic. A lumbar puncture was not performed because the patient's condition began to improve and there were pustules overlying the lumbar spine. The patient's illness was severe enough to consider using a systemic chemotherapeutic agent, but there was a dramatic response to idoxuridine in dimethylsulphoxide applied locally to the penile lesion and pustules. It is possible that prompt treatment with a systemic agent could have prevented infection of the sensory ganglia.

The result of the electron microscope examination of the biopsy specimen of the neck lesion is of interest in that no intracellular filaments or microtubules were found. Filament production has been associated with type 2 strains of virus growing in the chick embryo (Couch and Nahmias, 1969) and BHK cells (Smith *et al.*, 1973). Filaments have been described in HEp2 cells (Schwartz and Roizman, 1969), a cell line of human origin, but Cabral and

Schaffer (1976) failed to detect the structures in cultured human embryo lung cells. The viruses isolated from our patient did produce filaments when grown in BHK and HEp2 cells.

We thank Dr S. J. Urbaniak of the Regional Blood Transfusion Service for arranging the immunological assessment of the patient.

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Current approaches to the diagnosis, treatment, and reporting of trichomoniasis and candidosis

B. H. O'CONNOR* AND M. W. ADLER

From the Academic Department of Genitourinary Medicine, Middlesex Hospital Medical School, London

SUMMARY The current approach to the management of trichomoniasis and candidosis in sexually transmitted disease (STD) clinics in England and Wales is described. Microscopy alone was used in the diagnosis of trichomoniasis in 44% of clinics and of candidosis in 35% of clinics. Oral metronidazole was used for the treatment of trichomoniasis in women in 92% of clinics. Vaginal pessaries containing nystatin or clotrimazole were routinely used to treat candidosis in 95% of clinics. Male sexual contacts of female patients with candidosis and trichomoniasis were invited to attend for examination in 88% of clinics. Physicians in 81% of clinics prescribed treatment on epidemiological grounds for male contacts of female patients with trichomoniasis. A more uniform approach to the diagnostic categories used for the quarterly returns for cases treated epidemio-logically is recommended.

Introduction

Trichomoniasis and candidosis rarely exhibit the more serious consequences associated with some of the other sexually transmitted diseases (STDs). The two conditions, however, are common and together account for nearly as many notified cases as does gonorrhoea (Department of Health and Social Security, 1976).

Previous papers in this series have described a survey of the facilities and services provided by STD clinics in England and Wales (Adler *et al.*, 1978a), and the diagnosis, treatment, and notification of certain diseases, including gonorrhoea (Adler, 1978b, c), herpes genitalis (Belsey and Adler, 1978), and non-specific genital infection (Adler, 1978d, e).

This paper describes the current approach to the management of trichomoniasis and candidosis by physicians working in 173 clinics for female patients and in 171 clinics for male patients.

Results

TRICHOMONIASIS

Diagnosis

Physicians working in 77 (44%) clinics diagnosed trichomoniasis in female patients by microscopical

examination of vaginal secretions alone. In a further 93 (54%) clinics, cultures of vaginal samples were also used, and finally in three clinics cultures were used alone. The commonest culture medium used was Feinberg-Whittington medium, or one of its modifications (in 69 clinics, 72% of those using cultures).

Although physicians in all clinics had access to a microscopy service, in eight clinics the specimens for trichomoniasis had to be sent to a central laboratory and the results were not available at the patient's initial attendance. Treatment was instituted on the basis of the history and clinical findings by physicians in six of these clinics and in one where the clinical history and examination was suggestive of salpingitis. In one clinic treatment was given only after positive microscopical examination results were received from the laboratory.

Examination of male contacts

Physicians in 150 clinics (88% of those seeing male patients) attempted to see male sexual contacts of women with trichomoniasis; in 112 clinics this was routine practice for all contacts. In the remaining 38 clinics sexual contacts were seen in only selected instances, the commonest reasons being that the woman had recurrent attacks of trichomoniasis, that the man was the regular sexual partner, or that the man had symptoms.

Male contacts of female patients with trichomoniasis may be asymptomatic and may have no

*Present address: Camden and Islington Area Health Authority (Teaching)

Address for reprints: Professor M. W. Adler, Academic Department of Genitourinary Medicine, Middlesex Hospital Medical School, London W1N 8AA

Received for publication 29 June 1978

urethral discharge on examination. In 126 (84%) of the 150 clinics where sexual contacts were examined physicians used a variety of methods to collect specimens in these circumstances. The most common method was to perform a urethral scrape (Table 1). Specimens were collected by one method only in 68 clinics, by two methods in 37 clinics, and by three or more methods in 15 clinics. Physicians working in 24 clinics did not take specimens for laboratory examination.

Table 1 *Methods of obtaining specimens from asymptomatic male contacts of female patients with trichomoniasis*

Method	Clinics (n=126)	
	No.	%
Urethral scrape	105	83.3
Prostatic smear	32	25.4
Specimen of centrifuged urine	28	22.2
Early morning smear	15	11.9
Threads from two-glass urine test	10	7.9
Other	1	0.8
Not known	6	4.8

Epidemiological treatment

In the survey epidemiological treatment was defined as treatment given to named sexual contacts after a history of exposure but without (or in advance of) confirmatory pathological findings. Physicians working in 138 (81%) clinics were prepared to use this approach for male sexual contacts. In 122 clinics specimens were taken for laboratory examination: for microscopy in 58 clinics, for both microscopy and culture in 60 clinics, and for cultures alone in four clinics. In 16 clinics, physicians examined the male contact but did not carry out any investigations before prescribing treatment.

Treatment by proxy

In contrast to epidemiological treatment physicians in 79 (46%) clinics prescribed treatment for a male contact in his absence and gave the female patient tablets for him. This constitutes treatment by proxy, which was used on all occasions by physicians working in 24 clinics and only occasionally in the remaining 55 clinics.

Antimicrobial treatment for trichomoniasis

The treatment usually prescribed for trichomoniasis was oral metronidazole; this was used in 160 (92%) clinics for women and in 156 (91%) clinics for men. The commonest regimens for female patients (Table 2) were 0.6 gm daily for seven days (88 clinics) and 0.8 gm daily for five days (26 clinics). In only seven clinics was this drug prescribed for

Table 2 *Drugs used to treat trichomoniasis in women*

	Number of clinics					
	Duration of treatment regimen (days)					
Drugs (grams/day)	1-1½	2-3	5-6	7	10	Total
Metronidazole						
0.4	—	—	1	—	—	1
0.6	—	—	1	88	2	91
0.8	—	5	26	11	—	42
1.2	2	—	—	2	5	9
1.6	—	7	—	—	—	7
2.0	9	1	—	—	—	10
Total no. of clinics	11	13	28	101	7	160
Nimorazole						
1.0	3	3	—	—	—	6
2.0	6	—	—	—	—	6
2.5	1	—	—	—	—	1
Total no. of clinics	10	3	—	—	—	13

longer than seven days—1.2 gm daily for 10 days (five clinics) and 0.6 gm daily for 10 days (two clinics). The pattern of treatment for men was similar. Physicians working in 13 clinics for women and in 16 (9%) clinics for men prescribed nimorazole, the most usual regimen being a single dose of 2 gm.

Alcohol and treatment

Consultants working in 111 and 108 clinics for male and female patients respectively advised patients not to drink alcohol during treatment. The reasons are shown in Table 3; the commonest reason given (70 clinics) was the possible disulfiram-like ('antabuse') effect with treatment. In 92 clinics physicians gave one reason, in 12 they gave two, and in five they gave three.

Table 3 *Reasons given for asking patients to abstain from alcohol during treatment for trichomoniasis*

Reasons given	Clinics (n=111)	
	No.	%
Disulfiram-like (antabuse) effect	70	63.1
Irritates urethra/exacerbates urethritis	19	17.1
Patients should remain sober	19	17.1
Delays drug absorption	12	10.8
Causes pelvic congestion	5	4.5
Standard practice	4	3.6
Causes relapse	2	1.8
Not known	2	1.8

Follow-up investigations

The pattern of follow-up investigations (Table 4) shows that physicians working in 128 (74%) clinics for women and in 72 (42%) clinics for men repeated investigations on two or more occasions. No follow up was carried out in seven clinics for women nor in 50 clinics for men.

Table 4 *Number of repeat investigations after treatment for trichomoniasis*

No. of repeat investigations	Clinics			
	For women		For men	
	No.	%	No.	%
None	7	4.0	50	29.2
One	38	22.0	42	24.6
Two	82	47.4	45	26.3
Three or more	46	26.6	27	15.8
Not known	0	0.0	7	4.1
Total	173	100.0	171	100.0

Quarterly returns

Consultants were asked to indicate the criteria which they used to make a return for a case of trichomoniasis to the Department of Health and Social Security (DHSS). All physicians using positive microscopical findings alone to make a diagnosis of trichomoniasis would use this as a basis for notification. In those clinics where microscopy and culture were both used, a case would be notified if either or both gave positive results. In eight clinics treatment was given in all or selected cases on clinical grounds alone. In three of the clinics a return would be made on the basis of the clinical diagnosis without supportive investigations.

Physicians treating male contacts epidemiologically or by proxy used different diagnostic categories. Table 5 shows the categories used when the smears or cultures or both gave negative results and the patient had either already been treated (cases treated epidemiologically) or had not been seen in the clinic (cases treated by proxy). Physicians working in 101 clinics notified the DHSS (Table 5) of male contacts treated epidemiologically as 'other conditions requiring treatment' (D2) and in 22 clinics as trichomoniasis (C6). Consultants in nine clinics notified male contacts treated by proxy

Table 5 *Returns to the DHSS for male contacts treated for trichomoniasis in whom laboratory investigations gave negative results*

Categories used in returns	Cases treated epidemiologically		Cases treated by proxy	
	No.	%	No.	%
Trichomoniasis (C6)	22	15.9	0	0.0
Non-specific genital infection (C4)	7	5.1	0	0.0
Other conditions requiring treatment (D2)	101	73.2	7	8.9
Other conditions not requiring treatment (D3)	8	5.8	2	2.5
No return	0	0.0	62	78.5
Not known	0	0.0	8	10.1
Total	138	100.0	79	100.0

either as 'other conditions requiring treatment' (D2) or as 'other conditions not requiring treatment' (D3). In most instances no return was made for cases treated by proxy.

CANDIDOSIS

Diagnosis

In 61 (35%) clinics physicians made the diagnosis of candidosis in female patients on the basis of microscopical examination of vaginal secretions alone. In the remaining 112 (65%) clinics cultures of vaginal material were also used, the commonest medium being Sabouraud's or one of its modifications (Table 6).

In nine clinics smear results were not available at the patient's first attendance, and physicians in seven of these clinics treated the patient for candidosis on the basis of clinical history and examination.

Table 6 *Culture media used for diagnosis of candidosis*

Culture medium	Clinics (n=112)	
	No.	%
Sabouraud or modification	56	50.0
Feinberg-Whittington or modification	31	27.7
Thayer-Martin or modification	17	15.2
Transport media	2	1.8
Other	3	2.7
Not known	3	2.7
Total	112	100.0

Examination of male contacts

In 150 clinics sexual contacts of female patients with candidosis were invited to attend—in all instances, in 49 clinics, or, in selected instances, in 101 clinics. The commonest reasons for selection (Table 7) were a history of symptoms in the male contact or recurrent attacks in the original female patient. One reason for selection was given in 74 clinics two in 25 clinics, and three in two clinics.

Table 7 *Reasons given for examination of selected male contacts of female patients with candidosis*

Reasons given	Clinics (n=101)	
	No.	%
History of symptoms in man	78	77.2
Recurrent attacks in woman	36	35.6
Regular partner	9	8.9
Patient's/contact's wish	6	5.9
Promiscuity of female partner	1	1.0

Epidemiological treatment and treatment by proxy
Physicians in 56 (33%) clinics used epidemiological treatment for male contacts, although in nine of these no investigations were carried out to establish the diagnosis. Treatment by proxy was the usual or occasional practice in 41 (24%) clinics.

Antifungal treatment

The commonest form of treatment for candidosis was pessaries; these were used in 164 (95%) clinics. Table 8 shows the types of pessaries and the regimens used; the most commonly used were nystatin—two a day for 14 to 15 days (32 clinics)—or clotrimazole—one daily for six to seven days (27 clinics). Miconazole pessaries were prescribed in nine clinics and hydrargaphen pessaries in three clinics.

Table 8 Number of clinics using pessaries to treat candidosis

Type of pessary	Duration of treatment (days)	Number of pessaries per day			Total no. of clinics (n=164)
		One	Two	Not specified	
Nystatin	6-7	11	11	2	24
	10	3	15	7	25
	14-15	16	32	6	54
	20-21	3	0	2	5
Total no. of clinics		33	58	17	108
Clotrimazole	6-7	27	0	7	34
	9	2	0	0	2
	12	6	2	0	8
Total no. of clinics		35	2	7	44
Other treatment					12

Most physicians did not treat every patient who was diagnosed as having candidosis. In 91 (53%) clinics they were selective (Table 9). The commonest reasons for giving treatment in selected instances were that the patient had symptoms or signs. In 41 clinics one criterion for selection for treatment was given, in 40 two criteria were given, and in seven three or more were mentioned.

Follow-up investigations and second treatment

In 154 (89%) clinics at least one follow-up investigation was carried out after treatment had been prescribed. If treatment appeared to have failed physicians in 137 (79%) clinics prescribed a second course of pessaries; in 52 of these clinics a course of nystatin tablets was also prescribed.

Other measures were taken in some clinics if the patient appeared refractory to the first course of

Table 9 Reasons given for treatment selection of women with candidosis

Reasons given	Clinics (n=91)	
	No.	%
Symptoms	83	91.2
Signs	23	25.3
Positive microscopical findings	12	13.2
Presence of candidosis might be masked by other factors, such as pregnancy or antibiotic treatment	10	11.0
Positive microscopical evidence in the absence of symptoms	7	7.7
Symptoms in male consort	5	5.5
Recurrent disease in female patient	3	3.3
Anxiety in patient	2	2.2
Not known	3	3.3

treatment: in 10 clinics diabetes mellitus was excluded and in three clinics a full medical examination was performed in an attempt to identify any predisposing factors. In five clinics, the patient was recommended to stop taking oral contraceptives.

Quarterly returns

In the 112 clinics where the diagnosis was made on the basis of both microscopical evidence and culture, a return was made for candidosis (C7) if they both gave positive results. Physicians in 104 (93%) of these clinics would make a return of candidosis on the evidence of microscopy even if the culture results were negative; in 95 (85%) of these clinics a return was made for candidosis if the results were negative on microscopical examination but positive on culture. In all clinics where microscopy was the only diagnostic criterion, microscopical evidence would be used as a basis for notification if the results were positive.

Physicians treating patients with candidosis on clinical evidence alone (seven clinics) reported all these as cases of candidosis. Of the physicians working in 56 clinics who treated patients epidemiologically, only those in six clinics recorded these as cases of candidosis. In the remaining 50 clinics such cases were included under the category of 'other conditions requiring treatment' (D2). In four clinics physicians would make returns for male contacts treated by proxy, in one clinic as candidosis (C7) and in three clinics as 'other conditions requiring treatment' (D2). In 27 clinics no returns were made in these instances. The type of return was not ascertained from physicians in 10 clinics.

Physicians were asked whether they made returns to the DHSS for all cases of candidosis. In 115 clinics each case diagnosed was returned but in 55 clinics returns included only cases which were both

diagnosed and treated. In three clinics only cases in which candidosis was the principal diagnosis and had been treated were included in the returns.

Discussion

The diagnosis of trichomoniasis and candidosis depends on both clinical and laboratory examination. Feinberg and Whittington (1957) noted that trichomonads were detected in an additional 18% of vaginal specimens if culture techniques were used in association with microscopy. King and Nicol (1975) recommended the combination of smear and culture techniques as being more effective in establishing a diagnosis than either one alone. Recent work suggests that the diagnosis of candidosis may be missed more frequently than that of trichomoniasis if culture techniques are not used in combination with microscopy. Eddie (1968) showed that this could occur in up to 50% of cases, and Becker and Schweisfurth (1971) reported that 60% of candidal infections would be missed if microscopy was used alone. It has been suggested (Oriol, 1977) that culture of specimens is essential for a definitive diagnosis. Physicians working in 44% and 35% of clinics made the diagnosis of trichomoniasis and of candidosis respectively on microscopy alone; thus the use of culture techniques in addition to microscopy is suggested. Physicians working in seven clinics treated trichomoniasis and candidosis without any confirmatory microscopical or cultural evidence, a course of action which presupposes that it is possible to differentiate between these two diagnoses on macroscopical evidence alone and exclude other coexisting STDs. This is unlikely, and the use of some confirmatory laboratory procedures (microscopy or culture or both) is essential.

A distinction exists between the asymptomatic individual who is carrying *Candida albicans* as a commensal, but is free from clinical disease, and the patient with symptoms and signs who has a clinical, parasitic infestation with *C. albicans* (Oriol, 1977). The criteria for this differentiation into commensalism or parasitism are poorly defined (Ridley 1976), and some clinicians consider that symptoms are an unreliable guide to diagnosis (Morton and Rashid, 1977). In our survey some physicians did not treat patients with candidosis even though it had been diagnosed. A few physicians felt that positive microscopical evidence was a suitable indication for treatment, although in the absence of symptoms and signs in such cases the organism could be considered saprophytic and was not treated by others of their colleagues.

The widespread use by physicians of epidemiological treatment for trichomoniasis partly reflects the difficulty of making a diagnosis in the male contact. Watt and Jennison (1960) investigated a group of male patients whose female partners had intractable trichomoniasis and found that the majority in whom cultures gave positive results were asymptomatic. In the present survey specimens from asymptomatic male contacts were collected in most clinics, a practice which might usefully be extended to the remaining clinics to assist in the diagnosis of trichomoniasis in the male contact.

Treatment by proxy—in which the female patient is given tablets for her male partner—obviously prevents any diagnosis or exclusion of the disease (because the male contact remains unexamined). Tsao (1969) noted in a series of patients that the proportion of women diagnosed as having trichomoniasis who also had *Neisseria gonorrhoeae* in their microscopy or culture specimens was 46%; recent reports have drawn attention to asymptomatic gonorrhoea in men (Portnoy *et al.*, 1974; Handsfield *et al.*, 1974). The taking of specimens from sexual contacts of women with trichomoniasis for laboratory examination is, therefore, important in making a full assessment in both partners. Any possibility of investigating a contact should not be lost, and the practice of giving treatment to patients who have not been seen by a doctor—and who are, therefore, not shown to be suffering from a disease and who might be affected by another concurrent STD—should be discouraged.

Metronidazole selectively inhibits parts of the alcohol detoxification pathway and has, therefore, a therapeutic role in the treatment of alcoholism in combination with disulfiram ('antabuse') (Rothstein and Clancy, 1969) or alone (Madalena and de Mattos, 1967). This metabolic interaction, which causes nausea and dizziness, is an important and uncomfortable side effect when patients receiving metronidazole take alcohol. Physicians in 60% of clinics did not specifically mention this to the patient. If these side effects appear without warning the patient may consider his reaction to the drug to be idiosyncratic and therefore stop his course of treatment.

Male contacts of women with trichomoniasis or candidosis treated epidemiologically in many clinics are usually notified to the DHSS as 'other conditions requiring treatment' (D2). Physicians recorded a definitive diagnosis of trichomoniasis (C6) and candidosis (C7) on a presumptive contact history alone without subsequent laboratory confirmation in 22 and six clinics respectively. The situation is further complicated by the inclusion in DHSS statistical results of cases of trichomoniasis and

candidosis which have been diagnosed on clinical evidence alone. In addition, physicians working in nine clinics made returns to the DHSS for sexual contacts of trichomoniasis treated by proxy (D2 or D3) and in four for contacts of candidosis. Categorisation in these ways will alter the picture of the true size of the problem. This underlines the necessity for standardisation of the returns by agreement concerning diagnostic criteria, by the indication of cases treated epidemiologically as a separate category, and by the exclusion from returns of individuals who do not attend the clinic.

This study was supported as part of a project grant from the Medical Research Council. We thank Miss E. M. Belsey for statistical assistance. We are most grateful to Dr R. D. Catterall and Professor D. L. Miller for their encouragement, support, and comments.

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A theory of the epidemiology of gonorrhoea

W. F. FELTON

From the Royal Sussex County Hospital, Brighton, Sussex

SUMMARY Gonorrhoea is a typical endemic disease. Its continuity has been traditionally attributed to the existence of a pool of infected promiscuous women. Alternative theories have suggested a dynamic approach which gives equal importance to the man and the woman. Models constructed to simulate the epidemiology of gonorrhoea have so far failed to be realistic. An hypothesis that the distribution of infections is limited by the number of opportunities for transmission presented by the pattern of sexual behaviour may explain the low prevalence of the disease in England.

Introduction

Gonorrhoea is an endemic disease which is distinguished by its continuity from year to year and from century to century. Before and after the introduction of penicillin sudden and unexpected local outbreaks of infection have been rare. The Chief Medical Officer's statistics from 1918 until the present time (Ministry of Health, 1918-63; Department of Health and Social Security, 1964-77) have shown upward and downward trends, but either way the trends have lasted a decade or longer. There have been no sporadic or erratic changes of direction. The annual variation in the number of cases recorded has been of the order of 10% except around the wartime peaks. The upward trend of the last two decades is slowing. Nevertheless, the apparent failure to influence the distribution of gonorrhoea to any marked degree by successful treatment and by diligent contact-tracing calls for an explanation.

There is a notable lack of epidemiological data and much of what are available—for example, the Chief Medical Officer's figures—have been regarded as unsatisfactory. The Chief Medical Officer's figures, however, do cover a continuous period of 60 years, during which they have shown a remarkable consistency. They would seem to provide a worthwhile base on which to continue the theoretical discussion begun by Colonel Harrison (1917), who pioneered the organisation of clinics and the collection of their statistics.

INFECTIOUS PROMISCUOUS FEMALE POOL

The difficulty of bringing contacts to diagnosis and treatment has beset clinicians since serious attempts

were made to bring gonorrhoea under control. Harrison (Ministry of Health, 1927) tried to measure this failure of discovery by comparing the relative incidence of syphilis and gonorrhoea in the armed Forces, where notification was compulsory, and in the civilian population, where it was not. In 1920 there were seven to 10 cases of gonorrhoea among Army, Navy, and Airforce personnel to one of syphilis, whereas among civilians the ratio of cases was more equally balanced at 2.5:1 for men and 1.4:1 for women (Ministry of Health, 1927). This was an unfortunate comparison, since 1920 was a record year for syphilis with no less than 42 805 cases. It is also clear now that the epidemiology of syphilis and gonorrhoea are not comparable, for the former has declined while the latter has increased in the same population.

In 1976 (Department of Health and Social Security, 1978) there were 3878 cases of syphilis and 58 725 cases of gonorrhoea in England. This is a ratio of 1:15.

The idea of failure to discover cases was further supported by the imbalance of men and women which in 1920 was 5.2:1 and until 1961 remained at nearly 4:1. This suggested that the failure mainly concerned women, and, as it is well known, a large proportion of these are asymptomatic. The male-to-female ratio for syphilis of 2.5:1 suggested that the gonorrhoea ratio would be lower if all cases were found and recorded. Harrison argued from this that there might have been more than 100 000 cases in the population in 1920 of which less than half were brought to treatment (Ministry of Health, 1927).

In 1976 the male-to-female ratio for gonorrhoea was 1.7:1 and for syphilis 3.2:1. The position has practically reversed itself since 1920. The change in the figure for syphilis is probably partly a reflection

Address for reprints: Dr W. F. Felton, Royal Sussex County Hospital, Brighton BN2 5BE

Received for publication 29 May 1978

of the greater involvement of male homosexuals. It can also be observed with hindsight that the male-to-female ratio remained constant at near 4:1 for 40 years. It seems hardly likely that all the efforts at contact-tracing and a revolution in treatment following the use of penicillin would have failed to make some impression. If the existence of the imbalance was mainly due to a failure to discover female cases a change might have been expected before 1961.

Today's authorities, still under the influence of this early theory, continue to write of an infectious female pool as though it is the key factor in the continuity of gonorrhoea (World Health Organisation, 1975). The basis for its existence has always been largely intuitive. It is perhaps more reasonable to suppose that the imbalance may be part of the natural history of the disease. There may always have been more male than female cases. If the mean infectious period in women is longer than that in men—a reasonable clinical proposition (Morton, 1972)—it is probable that women will infect more men than vice versa. If more men change partners more often than women, then it would follow that men are at greater risk and would acquire more infections.

There is no doubt that a reservoir of infectious men and women exists. In England the infectious female pool almost certainly does not consist of a group of stubbornly undiscoverable women. Willcox (1965a; 1966a, b, c; 1967), in the series of papers in which he develops his theory, points out that the pool is always losing and recruiting members. Recruitment is only possible with the aid of infectious men who are as important in the continuity of gonorrhoea as women. If this last point is taken, the analogy of the pool can be exchanged for that of a wave of infections in men and women passing through a population. The way is then open to propose a dynamic theory of gonorrhoea distribution.

New theories

Workers who have offered new ideas to explain endemic gonorrhoea have been confronted by a number of difficulties. The specialist epidemiologist looks for fields in which there is an existing or potential body of reliable data. It is clear that new data will be difficult and expensive to collect. Any new theories, like the old, will have to depend at first on existing data and clinical intuition.

Clinicians for their part have little time to read closely argued theoretical discussions which, when summarised, seem to offer little immediate hope of making their control task more effective. The

sophisticated mathematical approach may leave the clinician without any clear understanding of what is proposed. On the other hand, the infectious pool theory is easily grasped and has the authority of longevity on its side. It offers a target to be found and destroyed.

Willcox (1965a) first developed the argument for a dynamic approach without resort to difficult mathematics. His model led him to postulate that gonorrhoea was balanced on a knife edge and may increase or decrease exponentially from a point of equilibrium. The continuity of both local and national trends is evidence against the existence of such a precarious situation.

Lee *et al.* (1972) devised a simple model which was based on the assumption that gonorrhoea had been increasing in the USA at an exponential rate. They reason that this means that there is a 'positive feedback loop' or that an increase now will lead to an even bigger increase later. From this they deduce that a relatively small reduction in transmissions of say 10% which might be achieved by the use of an intravaginal antiseptic compound could bring gonorrhoea under control.

Gladys Reynold's (1973) model, which has been used and elaborated by St John (1973), employs, from the clinician's point of view, difficult mathematics. She, like Lee *et al.* (1972), concludes that relatively small changes such as the reduction of the duration of infections in women from 100 to 92 days would halve the prevalence of gonorrhoea in about three years. If the reduction was maintained the prevalence would continue to fall.

In the light of the recorded experience of the last 60 years the clinician who doubts the validity of these propositions can hardly be called a cynic. Commonsense suggests that the world-wide use of penicillin was a major event probably reducing the mean duration of infections in women. In no country has the introduction of penicillin brought about a collapse of the endemic level. It is true that there was a false dawn in England during the early 1950s. Events now point strongly to the low figure of 1955 being due to social factors, such as the stabilisation of the pattern of sexual behaviour.

Two other models have been constructed, one by Beral (personal communication) and the other by Constable (1975). They both employ rather more difficult mathematics. They might both be described as second generation as they bring the endemic situation into equilibrium. This is a great advance, since the reason why an infection is endemic is that it is in a state of equilibrium within its host population. The incidence and prevalence are shown as relatively stable and not subject to wide fluctuations on account of small changes in the parameters of

the models. The method of bringing about the equilibrium is, however, open to criticism and depends upon a sufficient number of infected men contacting infected women so that their respective infections are not reproduced. Clinical intuition finds this difficult to accept for a number of reasons, more especially because if it were true the seeking out and removal of infection might actually increase the incidence of disease.

The reason for casting doubt on the first two generations of new theory is to clear the ground for the third generation. The parameters must describe as faithfully as possible the pattern of sexual behaviour quantitatively in relation to time and space. On the microbiological side they must describe the characteristic infectious periods for the two sexes and the degrees of infectivity between the two sexes. It is well known, however, that there are few hard data to cover any of these matters and one reason for making a model is to try and fit some values into it. This experience can be helpful in the preparation of investigations and in determining priorities.

For the purpose of this part of the argument let us assume the following. Infectivity in either direction is of a high order, say 80%. Natural or acquired immunity is an unimportant factor and if it exists is very rare. In England the great majority of cases of gonorrhoea are treated and the characteristic infectious period for men is around 10 days and for women about 30 days. The long-standing undiscovered case of either sex is the exception rather than the rule.

The dynamics of gonorrhoea are provided by the pattern of sexual behaviour and the movement of men and women within the population. This behaviour and movement provides the network through which the gonococcus passes from person to person and place to place. The network has to be in existence before an endemic situation can develop. An example is the spread that followed the introduction of gonorrhoea into the island of Tahiti by French and English sailors (Hunter, 1786).

The essential and basic element of the pattern of sexual behaviour is the paired partner change by one person. The first change provides the opportunity to acquire and the second to transmit infection. The paired partner change will only provide the opportunity for transmission if the interval between the changes is within the characteristic period of infectiousness. Thus what the gonococcus needs for its survival is a series of linked partner changes, all of which are correctly timed for transmission. In a population of tens of millions partner changes must occur by the thousand every day,

but the probability that any one of those changes will be correctly linked in respect of time with a chain of other partner changes equally correctly linked is low. This explains why in a population of more than 45 000 000 in England the rate of discovery and eradication of gonorrhoea is limited to an average of 160 cases per day.

If the next generation of gonorrhoea models uses the limitation of opportunities for transmission as a means of demonstrating how infections are held in a position near to equilibrium the results will be more realistic. In particular, it will be possible to postulate a low rate of prevalence. The available evidence suggests that the prevalence in England is less than 1% (Silverstone *et al.*, 1974).

It has already been proposed that the analogy of the pool should be replaced by that of a wave passing through the population in time. Its height is mainly determined by the number of opportunities presented to the gonococcus for its transmission. These opportunities are governed by social factors and are largely outside the influence of medical control programmes. The length of the wave is determined by the duration of infections. The length is also a function of the height, that is, the total number of infections. The duration of infections is within the scope of medical control programmes; indeed their main objective is to secure the earliest possible removal.

Unhappily even if the control programme is executed with both skill and enthusiasm the cases seen at the clinics are from the trailing edge of the wave. It has to be so because action is initiated on the discovery of an infection, which has often been present long enough to allow transmission. While it seems unlikely that it will ever be possible to control gonorrhoea satisfactorily, until infection can be blocked by providing artificial immunity, our present methods should be respected for the results they have achieved. The first achievement is the reduction of the complications to a level at which they are comparatively rare. The second is the reduction in the number of infections by shortening their duration. It seems likely that the fall in the male-to-female ratio may be partly the result of control. Thirdly, it is possible to speculate that the definitive diagnosis after screening at a clinic and the subsequent tracing of contacts slows down—even for a short time—the partner-change rate of the sexual grouping involved. This may result in the breaking of the chain of infection in the group. If this is true it is one way in which clinics are influencing what is happening at the leading edge of the wave.

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Treatment failure with penicillin in early syphilis

A. J. H. GILES AND A. G. LAWRENCE

From the Department of Genitourinary Medicine and Venereology,
St Stephen's Hospital, London

SUMMARY A patient with primary syphilis had a recurrence of his penile chancre after generally acceptable penicillin treatment. Retreatment with a similar but extended penicillin regimen was successful. This is the first report of such a treatment failure.

Case report

HISTORY

A 32-year-old Spanish homosexual man presented at the venereal diseases clinic at St Stephen's Hospital on 10 March 1978. He had noticed a sore on his penis for one week, to which he had applied a proprietary cream (not identified but bought in England). He admitted to sexual exposure in Paris on 17 December 1977 with a casual, untraceable contact and again in London on 3 March with a friend. He denied passive anal intercourse and orogenital activity. The only relevant past history was an episode of hepatitis in 1972.

EXAMINATION

On examination the foreskin was retractile, and there was a classical Hunterian primary chancre on the dorsal surface of the shaft of the penis at the coronal sulcus. The lesion measured 1 cm across. There was a painless, discrete, palpable lymph node in the left groin, but there were no other clinical findings.

INVESTIGATIONS

The results of investigations were as follows:

Darkground examination

Many characteristic motile treponemes were seen on 10 March 1978.

Serology

The results of both the Venereal Disease Research Laboratory (VDRL) and fluorescent treponemal antibody absorption (FTA-ABS) tests were positive (VDRL test at a 1/2 dilution).

The *Treponema pallidum* haemagglutination (TPHA) and Reiter protein complement-fixation (RPCFT) tests also gave positive results. The results for HBs antigen and viral culture for herpes simplex virus were negative. *Neisseria meningitidis* was isolated from a throat culture.

MANAGEMENT AND COURSE OF ILLNESS

A diagnosis of primary syphilis was made and routine treatment with daily injections of 0.6 mega-units of aqueous procaine penicillin (Depocillin, Brocades GB Ltd) intramuscularly for 12 days was given. After the first injection the patient experienced a severe Jarisch-Herxheimer reaction. Treatment finished on the 21 March.

The patient attended the clinic 13 days later for his first follow-up blood test; he complained at this stage that his sore had not healed. He denied any sexual activity since 3 March. On examination, the site of the chancre was still distinctly visible and indurated. No darkground examination was carried out on this day. Three weeks later when the patient attended on 28 April it was clinically apparent that the patient again had a classical chancre 8 mm in diameter at exactly the same site as the initial lesion six weeks previously. There was a degree of sclerosing lymphangitis underlying the ulcer; this added thickness to the lesion when palpated. The patient described how the original sore had appeared to close over the surface but had remained tender and swollen as if 'pus remained inside'. The lesion then broke down two days later and fluid oozed from it. The patient had not received any other medication since his clinic treatment. The investigations on 28 April 1978 were as follows:

Darkground examination

Many characteristic motile treponemes were seen. For confirmation, a direct fluorescent treponemal antibody (FTA) test was performed at the Venereal

Diseases Reference Laboratory at the London Hospital. This showed numerous bright fluorescing treponemes with the characteristic morphology of *Treponema pallidum* together with numerous pus cells and very few bacilli.

Serology

The VDRL test gave positive results (no dilution), as did the TPHA and RPCFT tests. The FTA test gave positive results for IgG antibody but negative results for IgM antibody.

No abnormalities were found on chest x-ray examination. The full blood count (FBC) was normal, and the urine analysis gave negative results for glucose, protein, and bilirubin.

RETREATMENT

Retreatment was started on 28 April 1978 with the same penicillin regimen; again a Jarisch-Herxheimer reaction occurred. After a week of treatment the ulcer was smaller (5mm) and darkground examination gave negative results. After 10 days' treatment the ulcer was a great deal smaller but was still indurated, and a small central area remained raw and oozed serum. After 15 days' treatment the ulcer surface had healed, although it was still indurated. A repeat VDRL test gave positive results (no dilution). The patient last attended five days after finishing his treatment on the 19 May 1978. There was minimal induration (1 cm) and no sign of the ulcer beginning to break down.

Discussion

The WHO recommendations for treatment of primary and secondary syphilis include a regimen of daily injections of aqueous procaine penicillin G at a dose of 600 000 units for a total of eight to 10 days (total 4.8–6.0 megaunits) (Idsøe *et al.* (1972)).

The United States Public Health Service also includes this regimen in their recommendations for early syphilis treatment (US Center for Disease Control, 1968). This recommended schedule was originally based on unpublished data; however, reports of results of treatment are now available. Schroeter *et al.* (1972) report on the results of treating 41 patients with primary and secondary syphilis who were followed up for more than one year. The retreatment rate at the end of one year was 3.8% and at the end of two years was 10.2%. The retreatment rate is a useful term in that it is not necessary to distinguish reinfections from treatment failures, often an impossible task. A recent report by Fiumara (1977a, b) gives a cure rate for treatment of primary and secondary syphilis with benzathine penicillin injections as

100%. However, he excluded all patients who needed retreatment from his figures, hence the possibly unrealistic cure rate. In England penicillin regimens vary from clinic to clinic; it has become accepted, however, that a regimen of daily treatments is the ideal treatment.

It cannot be doubted that our patient had primary syphilis, a chancre, on 10 March 1978 and again on 28 April 1978. In this case we do not think that a secondary microbial infection is likely to have been a complicating factor. Herpes culture showed negative results and healing occurred, albeit gradually, with the second course of treatment without the addition of any other antibiotics. A chancre redux—that is, a recurrence of the chancre at the same site following apparent healing—was said to be less rare in pre-penicillin days (Willcox, 1964). A pseudo chancre redux, a gummatous recurrence at the site of the original primary lesion, was excluded by finding live *Treponema pallidum* organisms (Hardy *et al.*, 1970).

The following are points which suggest a treatment failure:

1. The chancre recurred at the identical site.
2. The chancre was seen by one of us 13 days after the end of the first course of treatment (this is a shortish incubation period for syphilis).
3. During the second course of treatment it was noted that after only 10 days of treatment the ulcer had not healed over and was still oozing from a central erosion.
4. After treatment of primary syphilis there is thought to be a variable period of partial immunity to reinfection. Experimentally it is difficult to produce a primary chancre by inoculation during this period (Schofield, 1975).
5. Lastly, although it is difficult to accept, the patient absolutely denied any further sexual exposure. Needless to say this aspect was repeatedly investigated and must remain in question. (We were convinced that the patient would readily have admitted to a possibility of reinfection.)

To establish that nothing untoward had happened in the management of this patient, thorough discussion with all personnel concerned merely confirmed that the patient attended regularly for daily injections; this brought to light that the care with which penicillin doses were calculated and administered by the nursing staff was exemplary and they are to be congratulated.

There are two points which could suggest reinfection. Firstly, there was a gap of four weeks after the first treatment and before the second diagnosis was

made; and, secondly, there was a skin lesion remaining from the first chancre through which reinfection could have occurred and so produced an ulcer at the same site.

Conclusion

We do not consider there is evidence in this case to show that this was an infection with a treponeme which was less sensitive to penicillin. An extended course of treatment had produced what appears to be a satisfactory cure. The experience of this case indicates that there is no place for complacency in the treatment of early syphilis, and it re-emphasises what has been stressed by all preceding generations of syphilologists—the need for full surveillance after treatment.

In this case the possible reason for the failure of the initial treatment may have been the intense cell-mediated immune response which occurred, producing a primary chancre with marked fibrous infiltration and sclerosing lymphangitis locally; the amount of fibrous tissue possibly allowed an area of tissue to be incompletely penetrated by treponemacidal penicillin levels. Indeed, the patient stated that although the surface had almost healed by the end of the initial treatment the area remained

very swollen and hard, and this area subsequently broke down to produce the relapsed lesion.

Failure to cure syphilis with routine penicillin treatment has been reported before in a case of congenital syphilis (Hardy *et al.*, 1970). This is the first report, however, of an almost unequivocal treatment failure in primary syphilis with a generally acceptable treatment regimen.

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Correspondence

TO THE EDITOR, *British Journal of Venereal Diseases*

Inhibition of gonococci by a selective medium: disparity between isolates from sexual partners

Sir,

A proportion of clinical isolates of *Neisseria gonorrhoeae* fail to grow on selective medium containing vancomycin, colistin, nystatin, and trimethoprim (VCNT). Reyn and Bentzon (1972) and Brorson *et al.* (1973) reported the isolation frequency of these strains to be about 4% and 10% respectively and found vancomycin to be the inhibitory component. The sensitivity of these strains to vancomycin has been attributed to env mutations which result in phenotypic hypersensitivity to antibiotics including vancomycin (Sparling *et al.*, 1976). Observations in this department suggest that other mechanisms may also be involved in the inhibition of certain gonococcal isolates by vancomycin.

The isolation and identification of gonococci have been previously described (Platt, 1976a). Ready-poured bi-plates, containing GC selective medium (VCNT) and GC non-selective medium, were obtained from Gibco-Biocult (Paisley, Scotland) and each batch was quantitatively quality-controlled to ensure consistent inhibitory activity. The isolation frequency of VCNT-sensitive gonococci remained constant at between 4% and 6%.

Gonococci were isolated from 32 patients attending the department of genitourinary medicine at the West London Hospital. From four (25%) patients and their respective sexual partners gonococci were isolated which grew on non-selective medium but failed to grow on selective medium on both primary isolation and subsequent subculture. Gonococci isolated from a further 12 patients failed to grow on selective medium on primary isolation; of these six were male and six were female.

On subculture each of five isolates tested produced colonies on selective medium which were similar in size and number to a control plate lacking VCNT. The gonococci isolated from each of the 12 sexual partners grew well on selective medium on primary isolation.

Reyn and Bentzon (1972) found that about 40% of strains, inhibited by vancomycin on primary isolation, were capable of growth on subculture if a heavy inoculum was used, and they attributed this result to random variation in the original inocula and a 'training effect' during laboratory subculture. The high proportion (75%) of disparate vancomycin sensitivity between sexual partners on primary isolation and the resistance of the vancomycin-sensitive isolates on subsequent subculture suggests that an alternative mechanism is involved.

Although env mutations have been demonstrated in clinical isolates of *N. gonorrhoeae* (Eisenstein and Sparling, 1978), for vancomycin sensitivity to be solely under env control would require both isolates from sexual partners to exhibit similar sensitivity to VCNT. The results obtained from four pairs of patients are compatible with this hypothesis. The disparate results obtained with 24 paired isolates may be explained as a function of the physiological state of the organism *in vivo*. For many organisms the immediate history of an inoculum affects the response to inhibitors (Farwell and Brown, 1971; Brown, 1977). The physiological state of gonococci determines their response to CO₂ deprivation (Platt, 1976b). Thus it seems likely that the physiological state of gonococci *in vivo* may predispose certain strains to vancomycin sensitivity on primary isolation.

Yours faithfully,
D. J. Platt* and Anne Gerkent

*Department of Bacteriology and Immunology,
Western Infirmary,
Glasgow G11 6NT

†Department of Medical Microbiology,
Charing Cross Hospital,
Fulham Palace Road,
London W6

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TO THE EDITOR, *British Journal of Venereal Diseases*

Treatment of condyloma acuminatum with 5% 5-fluorouracil

Sir,

Podophyllin is still widely used in the treatment of condyloma acuminatum since its introduction in 1944, but the results are poor especially with multiple condylomata acuminata, when local side effects may be troublesome. Good results were obtained with 5-fluorouracil cream in the treatment of genital warts and the side effects were minimal (Handojo and Pardjono, 1973; Hayes, 1974; Dretler and Klein, 1975; Von Krogh, 1976). It was decided to see whether the results reported by these authors could be confirmed.

All men with condylomata acuminata were treated with 5% 5-fluorouracil cream until the condylomata were eradicated, usually after three to seven days. Patients were seen weekly for four weeks and followed up for three months. At each visit the distribution, number, and size of the warts were assessed and side effects recorded.

Forty-one male patients aged 15 to 39 years with condylomata acuminata of 15 to 60 days' duration, some of whom

had been previously treated with podophyllin, entered the study. Of these, 35 showed complete regression; 12 had skin condylomata, nine mucosal, nine skin and mucosal, three perianal, and two anal. The six patients who showed no reaction whatsoever all had mucosal condylomata and no reaction whatsoever occurred in these patients following the application of the 5-fluorouracil cream. The condylomata were eradicated in three to seven days.

Erythema, blistering, and superficial ulceration occurred in four patients and were controlled with the local application of copper sulphate. Eleven patients complained of slight erythema and one of a stinging sensation.

It is therefore considered justifiable to use 5-fluorouracil cream in all patients with genital warts under careful medical supervision.

Yours faithfully,

S. Pareek

27 Ascot Drive,
Great Sutton,
South Wirral,
Cheshire

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TO THE EDITOR, *British Journal of Venereal Diseases*

Campylobacter species in male homosexuals

Sir,

There is growing awareness of the importance of *Campylobacter* species in the aetiology of mild diarrhoea (*British Medical Journal*, 1978). This appeared relevant to us in view of the reports of a high incidence of enteric pathogens in homosexuals in the United States (Drusin *et al.*, 1976; Meyers *et al.*, 1977; Vaisrub, 1977).

Following the unexpected growth of *Campylobacter* species on a routine specimen for culture for *Neisseria*

gonorrhoeae taken from a heterosexual man, who attended this department complaining of rectal bleeding, a prospective study of homosexual men was undertaken to see if there was any relation between *Campylobacter* species and anal intercourse.

Fifty consecutive male homosexuals from whom rectal specimens had been cultured for *N. gonorrhoeae*, were investigated. Routine charcoal swabs placed in Stuart's medium were plated on to Oxoid Base no. 2 containing 7% lysed horse blood, vancomycin 10 µg/ml, polymixin B 2.5 IU/ml, and trimethoprim 5 µg/ml (final concentrations). Plates were incubated at 40°C in an atmosphere of about 5% oxygen and 10% carbon dioxide in hydrogen in an anaerobic jar without catalyst. The plates were examined at 18 and 48 hours (Communicable Disease Surveillance Centre, unpublished report). *Campylobacter* species were not isolated from any rectal sample. It is concluded that *Campylobacter* species are uncommon organisms in male homosexuals attending this clinic.

Yours faithfully,

P. D. Simmer.*

S. Tabaqchali†

Departments of *Genital Medicine and
†Medical Microbiology,
St Bartholomew's Hospital,
London EC1A 7BE

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TO THE EDITOR, *British Journal of Venereal Diseases*

Possible suppressor T lymphocyte activity in experimental syphilis

Sir,

In relation to the comments of Pavia *et al.* (1978) on the possible role of suppressor thymus-derived lymphocytes during

syphilis, we would like to report the following experiment.

Inbred CBA mice were used and fed on autoclaved water and an antibiotic-free diet. Mice aged 6 weeks were infected intraperitoneally with 0.5 ml of a suspension of *Treponema pallidum* (Nichols strain) adjusted to 0.5×10^6 spirochaetes. The preparation of the suspensions of *T. pallidum*, the method of tissue homogenisation, and the measurement of treponemal immunofluorescent antibodies have previously been described (Wright *et al.*, 1974; Wright and Wharton, 1977).

In the first experiment 24 mice were infected and six were left uninoculated. In the second experiment 27 mice were infected, six were inoculated intraperitoneally with a heat-killed suspension of *T. pallidum*, and six were again left uninoculated. Cyclophosphamide (Wyeth Brothers, Maidenhead) 150 mg/kg was given intraperitoneally at weekly intervals from the end of the second week to the seventh week following infection. In the first experiment all but three infected mice were given cyclophosphamide and in the second all but six. In both experiments there was a group of six mice that received only cyclophosphamide. Plasma was examined from all the mice before inoculation and at weekly intervals from the third to the seventh week following inoculation. The livers, spleens, kidneys, and hearts were taken from three cyclophosphamide-treated animals at weekly intervals and homogenised. The supernatants were then examined for the presence of spirochaetes.

All plasma from the infected control mice gave an immunofluorescent titre of >625 at the seventh week indicating that infection had taken place. No antibodies or spirochaetes were found in plasma from any other mice. No spirochaetes were seen in the supernates from homogenised tissues on darkground examination with the exception of three spirochaetes seen in the supernate of one liver homogenate taken on the third week from one of the mice in the first experiment.

The experiments indicate that cyclophosphamide does not enhance the multiplication of *T. pallidum*, and more surprisingly the infection is controlled in the absence of detectable antibody. It is relevant that in similar experiments in which some mice treated with one injection of cyclophosphamide 150 mg/kg and infected with *Borrelia duttoni* (STIB 503)

not only recovered from the infection in the absence of detectable antibody but the surface antigen changed in the first relapse (Wright and Frost, 1978). It is possible that the absence of borrelial, lytic, agglutinating, and spirochaetal fluorescent antibodies need not mean that all antibody is removed. Antibody which might induce or modulate the antigenic variation could still be present. It is conceivable that the production of hypothetical antigen-modulating-antibody might be enhanced by a deletion of T lymphocyte suppressor effect. Damage caused by cellular or humoral immune mechanisms would thereby be controlled and the microbial persistence ensured.

In unpublished experiments with Professor John Turk, cyclophosphamide 150 m/kg was given to rabbits 10 days

before intravenous *T. pallidum* inoculation. In the cyclophosphamide-treated rabbits lesions on the shaved, dorsal rabbit skin were exacerbated and the subsequent specific antibody titres were raised in these animals. Since spirochaetes were found only in the lesions and not in the unaffected tissue, the implication again is that there was an inhibition of T lymphocyte suppressor response rather than an increased effector cell activity.

Yours faithfully,

D. J. M. Wright and D. J. Frost

Department of Microbiology,
Charing Cross Hospital Medical School,
Fulham Palace Road,
London W6 8RF

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Notice

International Symposium on Sexually Transmitted Diseases

An international symposium will be held in conjunction with the first Pan American Congress of Andrology, 13-16 March 1979, in Caracas, Venezuela. The purpose is to initiate international co-operation and exchange of scientific information on current diagnosis and therapy of male diseases. Abstracts are accepted on urethral microbiology, gonococcal urethritis, aetiology and treatment of epididymitis, penile lesions, and genital herpesvirus; diagnostic tests for STD. Deadline: 1 December 1978. Programme director: F. N. Judson, M.D., Disease Control Service, 605 Bannock Street, Denver, Colorado 80204, USA (303) 893-7051.

Annual Report based on the Secretary's report to the Annual General Meeting on 27 October 1978

Medical Society for the Study of Venereal Diseases

During the past session, 53 new members were elected to the Society, of whom 33 are resident in the United Kingdom. Three members died and two resigned. It was found necessary to stop sending the *British Journal of Venereal Diseases* to 198 members because of underpayment of subscriptions, but their names were not removed from the list of members. This means that until action was taken about one-third of the membership of the Society was receiving the *Journal* without having paid for it properly, a situation which could not be allowed to continue. Since this action I am glad to report that many have adjusted their subscriptions to the correct level. At present the membership stands at 619; in October 1977 it was 570.

Five ordinary meetings were held during the period October to April and they were all extremely well attended. It is very gratifying that this high standard of attendance has been so well maintained, an excellent indicator of the health of the Society.

The Spring Meeting was held in Leeds from 19 to 21 May 1978 and was very successful, for which thanks are extended to Dr M. A. Waugh, the local organiser, who put in so much hard work. Our meeting was followed by the Congress of the International Union against the Venereal Diseases and Treponematoses, many of our members staying on to participate.

The Harrison Lecture was delivered by Dr C. S. Nicol at St Thomas's Hospital on 16 June 1978 and was followed by a buffet supper—an enjoyable evening which was well supported.

The Council of the Society, under the chairmanship of the President, Dr E. M. C. Dunlop, held five ordinary meetings and one extraordinary meeting, all of which were well attended.

At the October meeting Dr L. Cohen produced a gavel and block, which he had had made and inscribed in Wales. This was paid for by the honorarium which had been returned to the Society by Professor T. Turner, the second Harrison lecturer.

At this meeting Dr C. S. Nicol, speaking as adviser to the Department of Health and Social Security, a post he was about to vacate, mentioned that a completely new clinic was to be furnished at St Stephen's Hospital, London, and that the first chair in venereology was to be established at the Middlesex Hospital, London.

During subsequent meetings Council agreed to invite Professor I. Phillips and Dr E. Stolz to join the Editorial Committee of the *British Journal of Venereal Diseases*, and Dr R. D. Catterall announced that he had been asked to become the next adviser to the Department of Health and Social Security. It was agreed that the January 1979 ordinary general meeting should start at 7.00 p.m. to see if this would make attendance easier for provincial members. It was also agreed that recorded telephone messages for use when clinics were closed would be of value, the content of the messages being dictated by local circumstances.

An extraordinary council meeting was held at the Middlesex Hospital on 28 July to discuss the proposed new consultant contract. Its possible ill effects on our specialty were recognised, and it was agreed that direct representation to the Royal Commission should be made, indicating the areas of our disquiet, it having been previously ascertained that such representation would be acceptable.

The Editor of the *Journal*, Dr R. N. Thin, and his assistant editors, Dr J. D. Oriel and Dr B. A. Evans, merit our congratulations on maintaining the high standard of papers and extracts under mounting difficulties of financial strictures and pressures within the British Medical Association. The new format of the *Journal* has met with general approval and so far the first three issues of Volume 54 show a slight increase in size on Volume 53, which was 17 pages smaller than Volume 52.

At the Editorial Committee meeting, which was attended by Dr S. Lock, Editor of the *British Medical Journal*, relief was expressed that Dr Thin had

agreed to continue in post and not to retire in October as had been his original intention. Dr Lock expressed concern over the loss of 33 subscriptions from the British Isles but stated that world wide the overall pattern was the same as that shown by other specialist journals. The new format of the *Journal* was discussed and the question of change of title was raised, but it was agreed that the title should remain unchanged. The relationship of the *Journal* to the British Medical Association was discussed, as was the composition of the Editorial Committee. Discussion on both these matters continues. Dr A. E. Wilkinson tendered his resignation from the Committee and Dr Thin expressed gratitude for all the work he had done over the years.

Since the last report of the Secretary of the MSSVD was prepared, the Specialist Advisory Committee on Venereology has met twice. Very few senior registrar posts remain to be visited, and it was noted that the JCHMT had set a deadline of June 1979, by which time all initial visits are to be completed. More than 40 posts have been visited and approved for senior registrar training. Since there was an obvious need to tailor the number of training posts to the expected consultant vacancies the Committee had approached the British Co-operative Clinical Group for help to compile as complete a list as possible of future consultant vacancies.

The Specialist Advisory Committee considered and accepted applications for enrolment from a number of trainees but noted that there are still some senior registrars who have yet to enrol. Applications from trainees without acceptable higher qualifications have been deferred pending reassurance from their heads of departments that the standards of their general medical training had been satisfactory. It was noted that the JCHMT would only recognise part-time trainees who worked at least half-time. All trainees appointed as consultants in the National Health Service or as specialists in EEC countries have been recommended for certificates of accreditation. Applications for accreditation from two doctors,

who were not trainees, were refused. The Specialist Advisory Committee felt that appointment committees for senior registrar posts in London teaching hospitals should contain at least one external assessor.

The European Union of Medical Specialties met in Liège on 5 and 6 May 1978, and Dr Duncan Catterall attended as our representative.

It would appear then that we can reasonably congratulate ourselves on a healthy and flourishing Society, which continues to expand both in the United Kingdom and abroad despite the many difficulties within the National Health Service and, more generally, in the present world-wide climate.

Finally, I must thank all those who have helped me during my first year in

what is becoming an even more onerous office, especially the President, Dr E. M. C. Dunlop, the Council, and my predecessor in office, Dr C. B. S. Schofield.

J. BARROW
Hon. Secretary

Abstracts

These selected abstracts and titles from the world literature are arranged in the following sections:

Syphilis and other treponematoses

(Clinical and therapy; serology and biological false-positive phenomenon; pathology and experimental)

Gonorrhoea

(Clinical; microbiology; therapy)

Non-specific genital infection

Reiter's disease

Trichomoniasis

Candidosis

Genital herpes

Other sexually transmitted diseases

Public health and social aspects

Miscellaneous

Syphilis and other treponematoses (Clinical and therapy)

A child with the nephrotic syndrome associated with endemic syphilis (case report)

F. B. LE ROUX AND A. J. VAN BUUREN (1978). *South African Medical Journal*, 54, 205-208

A coloured boy aged 21 months was admitted to hospital with diarrhoea, generalised oedema, enlargement of the liver and spleen, and ascites. The urine contained protein and blood. The serum protein level was 49 g/l with a raised IgM and depression of the complement fractions C3 and C4; liver function tests were normal. The VDRL test was positive at a dilution of 1/512. A renal biopsy showed a diffuse endocapillary proliferative glomerulonephritis with many immature glomeruli; no spirochaetes were seen by silver staining. The child recovered rapidly after treatment with penicillin.

Serological tests for syphilis (VDRL, TPHA, and FTA-ABS) on the child's parents and two siblings were all negative. A 5-year-old boy, who lodged in the house, and his cousin, who often acted as a nursemaid to the patient, both had strongly positive VDRL and treponemal tests. The findings support the diagnosis of an endemic, non-venereal, treponemal infection and are thought to be the first report of renal involvement in this condition.

A. E. Wilkinson

[Reprinted from *Abstracts on Hygiene*, by permission of the Editor.]

Late benign syphilis of skin

D. K. GOETTE AND C. E. PRESCOTT (1978). *Southern Medical Journal*, 71, 505-508

Syphilis (Serology and biological false-positive phenomenon)

Hemagglutination tests for syphilis antibody

H. W. JAFFE, S. A. LARSEN, O. G. JONES, AND P. E. DANE (1978). *American Journal of Clinical Pathology*, 70, 230-233

The performance of two commercially available *Treponema pallidum* haemagglutination test reagents was compared with that of the FTA-ABS, VDRL, and RPR tests. In one (TPHA-S) sheep cells were used and in the other turkey cells (TPHA-T). On sera from 935 patients who were considered not to have syphilis the tests for reagin gave 0.75% false-positive results, the FTA-ABS 1.3%, the TPHA-S 0.96%, and the TPHA-T none. In tests on sera from 68 patients with treated and untreated early and latent syphilis the TPHA-S proved as sensitive as the FTA-ABS except in untreated primary cases; the TPHA-T was considerably less sensitive and gave 47 reactive results compared with 63 with the TPHA-S test. Both tests had a similar reproducibility. Variations in sensitivity between different batches of TPHA-T reagents was noted. Because of this and its undersensitivity, the use of this test is not recommended until further data are available.

A. E. Wilkinson

[Reprinted from *Abstracts on Hygiene*, by permission of the Editor.]

In vitro* activity of cefuroxime against *Treponema pallidum

L. XERRI AND P. ORSOLINI (1978). *Journal of Antimicrobial Chemotherapy*, 4, 189

Gonorrhoea (Clinical)

Role of iron in disseminated gonococcal infections

S. M. PAYNE, K. K. HOLMES, AND R. A. FINKELSTEIN (1978). *Infection and Immunity*, 20, 573-580

Screening for gonorrhoea (Leading article)

Lancet, 1978, 2, 880

Gonorrhoea (Microbiology)

Studies on circulating gonococcal antibodies and antigens

G. KWAPINSKI, E. KWAPINSKI, AND C. J. WEBB (1978). *Canadian Journal of Microbiology*, 24, 109-111

One hundred human sera obtained from acute gonococcal disease and 100 sera from nongonococcal diseases or healthy persons were concentrated four times and examined for the presence of circulating gonococcal antigens and antibodies by means of a counterimmunoelectrophoresis (CIE) and delayed hypersensitivity assay. Antibodies reacting with cytoplasmic gonococcal antigens were detected by CIE in 92% of sera received from patients suffering from acute gonococcal disease. Gonococcal

- antigens were found in the concentrated sera of 82.3% of patients on the basis of dermal reactions observed upon injections of these sera into the skin of rabbits sensitised with disrupted gonococci: 51.8% of the patients' sera gave delayed hypersensitivity reactions in rabbits sensitised with cytoplasmic antigens of *Neisseria gonorrhoeae*. Control sera from healthy people and those with non-gonococcal diseases did not react with any of the preparations tested.

Authors' summary

Contribution of a TEM-1-like β -lactamase to penicillin resistance in *Neisseria gonorrhoeae*

S. BERGSTRÖM, L. NORLANDER, A. NORQVIST, AND S. NORMARK (1978). *Antimicrobial Agents and Chemotherapy*, 13, 618-627

Two β -lactamase-producing strains of *Neisseria gonorrhoeae* were studied. The substrate profile, molecular weight, and isoelectric point of their β -lactamases were similar to those of the TEM-1 enzyme produced by many Gram-negative bacilli. The gonococcal β -lactamase was cell-bound during exponential growth and was most likely located in the periplasm. Penicillin hydrolysis was efficient in intact cells, suggesting that the cell-bound β -lactamase was freely accessible to benzylpenicillin. Both β -lactamase-producing strains of *N. gonorrhoeae* contained an additional multicopy plasmid with a mass of 3.3-megadaltons (Mdal). A spontaneous penicillin-susceptible revertant lacked both β -lactamase activity and the 3.3-Mdal plasmid, providing evidence for plasmid-mediated penicillin resistance. During a shift from GC medium to rich MOPS medium, growth of the penicillin-susceptible revertant in contrast to that of the plasmid-carrying strain was markedly impaired, suggesting a physiological effect due to the presence of the 3.3-Mdal plasmid.

Authors' summary

Differences in susceptibilities of virulent strains and avirulent strains of *Neisseria gonorrhoeae* to antimicrobial agents

D. V. LIM (1978). *Antimicrobial Agents and Chemotherapy*, 14, 45-51

Virulent cells (T1 colony type) and avirulent cells (T4 colony type) of

Neisseria gonorrhoeae were grown in a defined liquid medium in the presence of different antimicrobial agents. Bacteria of T1 colony type were found to be more resistant than bacteria of T4 colony type to the inhibitory effects of specific concentrations of ampicillin (0.50 μ g/ml) and penicillin (0.01 μ g/ml). Bacteria of T4 colony type, however, were found to be more resistant to the effects of chloramphenicol (0.40 μ g/ml), erythromycin (0.10 μ g/ml), spectinomycin (10.00 μ g/ml), and tetracycline (0.30 μ g/ml). The differences in susceptibilities of these bacteria to the antibiotics investigated were not due to differences in growth rate or to differences in permeability. The data suggest that virulent gonococci and avirulent gonococci have unique properties that affect their susceptibilities to certain antimicrobial agents.

Author's summary

A view of multiple drug resistance in *Neisseria gonorrhoeae*

M. A. CHAN AND M. GOLDNER (1978). *Journal of Antimicrobial Chemotherapy*, 4, 39-46

Comparison of a radiometric procedure with conventional methods for identification of *Neisseria*

R. R. STRAUSS, J. HOLDERBACH, AND H. FRIEDMAN (1978). *Journal of Clinical Microbiology*, 7, 419-425

Neisseria gonorrhoeae: pathogenicity of colonial type 5

S. HAFIZ, R. JENNINGS, M. G. MCENTEGART, AND A. E. JEPHCOTT (1978). *Journal of Clinical Pathology*, 31, 437-438

Lipopolysaccharide (R-type) as a common antigen of *Neisseria gonorrhoeae*.

1. Immunizing properties
B. B. DIENA, F. E. ASHTON, A. RYAN, AND R. WALLACE (1978). *Canadian Journal of Microbiology*, 24, 117-123

Lipopolysaccharide (R-type) as a common antigen of *Neisseria gonorrhoeae*.

2. Use of hen antiserum to gonococcal lipopolysaccharide in a rapid slide test for identification of *N. gonorrhoeae* from primary isolates and secondary cultures
R. WALLACE, A. RYAN, B. B. DIENA, C. MALYSHEFF, AND M. B. PERRY (1978). *Canadian Journal Microbiology*, 24, 124-128

Conjugative plasmids in *Neisseria gonorrhoeae*

T. E. SOX, W. MOHAMMED, E. BLACKMAN, G. BISWAS, AND P. F. SPARLING (1978). *Journal of Bacteriology*, 134, 278-286

Plasmid-mediated chromosomal gene transfer in *Neisseria gonorrhoeae*

M. ROBERTS AND S. FALKOW (1978). *Journal of Bacteriology*, 134, 66-70

Identification and penicillinase testing of *Neisseria gonorrhoeae* from primary isolation cultures of modified New York City medium

H. YOUNG (1978). *Journal of Clinical Microbiology*, 7, 247-250

Detection of antibodies to *Neisseria gonorrhoeae* by counterimmunoelectrophoresis

J. A. SCHRODER AND H. A. GAFAAR (1978). *Health Laboratory Science*, 15, 15-21

Effects of selected inhibitors on electron transport in *Neisseria gonorrhoeae*

E. A. KENIMER AND D. F. LAPP (1978). *Journal of Bacteriology*, 134, 537-545

Characterisation of mitogenic activity elicited by *Neisseria gonorrhoeae* ribosomal fractions

B. R. BRODEUR (1978). *Canadian Journal of Microbiology*, 24, 579-585

Cell envelope of *Neisseria gonorrhoeae*: phospholipase activity and its relationship to autolysis

A. F. CACCIAPUOTI, W. A. WEGENER, AND S. A. MORSE (1978). *Infection and Immunity*, 20, 418-438

Identification of some basic extractable compounds produced by *Neisseria gonorrhoeae* and *Neisseria meningitidis* in a defined medium

J. B. BROOKS, D. S. KELLOGG, JR., G. CHOUDHARY, C. C. ALLEY, AND J. A. LIDDLE (1978). *Journal of Clinical Microbiology*, 7, 415-418.

Differential binding of penicillin by membrane fractions from penicillin-susceptible and -resistant gonococci
W. J. RODRIGUEZ AND A. K. SAZ (1978). *Antimicrobial Agents and Chemotherapy*, 13, 589-617

Gonorrhoea (Therapy)

Ampicillin therapy for pharyngeal gonorrhoea

J. M. DICAPRIO, J. REYNOLDS, G. FRANK, J. CARBONE, AND R. NISHIMURA (1978). *Journal of the American Medical Association*, 239, 1631-1633

Single-dose oral ampicillin trihydrate is ineffective for the treatment of pharyngeal gonorrhoea. An evaluation was made of the efficacy of extended oral ampicillin therapy. The regimen consisted of a single oral 3.5 g dose of ampicillin trihydrate and 1.0 g of probenecid on the first day followed by 500 mg of ampicillin trihydrate four times a day for each of the succeeding two days, for a total ampicillin trihydrate dose of 7.5 g. One or two follow-up cultures taken within 35 days of completion of therapy were obtained in 77 of the 101 patients treated. Positive test-of-cure cultures were reported in three persons, two of whom may have been reinfected. The failure rate was calculated to range between 1.3% (1/77) to 3.9% (3/77).

Authors' summary

Non-specific genital infection

Non-gonococcal urethritis in Hawaii

T. KUBERSKI, S. PERRY, R. OHYE, AND N. WIEBENGA (1977). *Hawaii Medical Journal*, 36, 103-108

Nongonococcal urethritis (NGU) currently accounts for about 75% of all the urethritis seen in men attending the Hawaii State Venereal Disease Clinic. A study group of 26 consecutive male VD clinic patients with NGU were examined for various possible aetiological agents. From urethral cultures, *Chlamydia trachomatis* was recovered from 11 (42%) patients, *Trichomonas vaginalis*, herpesvirus hominis, and *Neisseria gonorrhoeae* from one patient each. Epidemiological data from 109 patients with NGU and 107 with gonorrhoea were retrospectively reviewed to find any differences between the two forms of urethritis. The significant differences observed were: NGU patients tended to be older, Caucasian, to have a longer duration of symptoms before coming to the clinic, and to engage in sexual intercourse despite symptoms more frequently.

Authors' summary

Infection of the uterine cervix with *Chlamydia trachomatis*

J. D. ORIEL, A. L. JOHNSON, D. BARLOW, B. J. THOMAS, K. NAYYAR, AND P. REEVE (1978). *Journal of Infectious Diseases*, 137, 443-451

For identification of those variables in the history and in the clinical and routine laboratory examination that are most likely to indicate infection of the uterine cervix with *Chlamydia trachomatis*, 284 women attending a venereal disease clinic were studied, of whom 58 (20.4%) yielded the organism from the cervix. Women with chlamydial cervical infection showed no distinctive symptoms. Although associations were found between the presence of *C. trachomatis* and cervical discontinuity, purulent cervical exudate, and inflammatory changes in cervical cytology, these signs were not pathognomonic of chlamydial infection since they were also seen in some women infected with other micro-organisms. There was a significant relationship between the presence of antibodies, detected by immunofluorescence, and the recovery of *C. trachomatis* in cell culture. There were no associations between ethnic group, history of sexually transmitted disease, method of contraception, phase in the menstrual cycle, or numbers of sexual contacts and infection with *C. trachomatis*.

Authors' summary

Single-antigen immunofluorescence test for chlamydial antibodies

P. SAIKKU AND J. PAAVONEN (1978). *Journal of Clinical Microbiology*, 8, 119-122

A simple method is presented for producing large numbers of inclusions of *Chlamydia trachomatis* serotype L2 in cell cultures on slides for immunofluorescence antibody staining. Preliminary results with a total of 1241 human sera from different groups were consistent with findings by earlier methods: 82% of *Chlamydia*-positive men with nongonococcal urethritis had antibodies at titres of ≥ 8 , with a geometric mean titre (GMT) of 44.9; 68.5% of *Chlamydia*-negative men with nongonococcal urethritis had titres of ≥ 8 , with a GMT of 38.6; 27% of male blood donors had titres of ≥ 8 , with a GMT of 19.6; 95.0% of *Chlamydia*-positive women had titres of ≥ 8 , with a GMT of 80.3; 67.0% of *Chlamydia*-negative partners of men with nongonococcal urethritis had titres of

≥ 8 , with a GMT of 47.0; 50.2% of control women had titres of ≥ 8 , with a GMT of 27.7%; and 8.1% of children (aged 1 to 15 years) had titres of ≥ 8 , with a GMT of 17.8. *Authors' summary*

Incidence of tetracycline-resistant strains of *Ureaplasma urealyticum*

R. T. EVANS AND D. TAYLOR-ROBINSON (1978). *Journal of Antimicrobial Chemotherapy*, 4, 57-64

Ureaplasmas were isolated from 141 (53.4%) of 264 men suffering from non-specific urethritis. Fourteen (9.9%) of these isolates were resistant to oxytetracycline and minocycline and they belonged to various serotypes. An increased resistance to minocycline was induced in three of four minocycline-sensitive ureaplasmas by passing them *in vitro* in the presence of this antibiotic. They did not attain the degree of resistance shown by naturally occurring resistant strains and it was not possible to transfer resistance from these strains to those which were sensitive to minocycline. Naturally occurring tetracycline-resistant ureaplasmas and those in which resistance had been induced retained an unaltered susceptibility to erythromycin.

Authors' summary

Urethral infection of chimpanzees by *Ureaplasma urealyticum*

D. TAYLOR-ROBINSON, R. H. PURCELL, W. T. LONDON, AND D. L. SLY (1978). *Journal of Medical Microbiology*, 2, 197-202

Two strains of *Ureaplasma urealyticum* (T-strain mycoplasmas) were used to inoculate three adult male chimpanzees urethrally. Both strains had previously been shown to produce urethritis following human intra-urethral inoculation (Taylor-Robinson *et al.*, 1977).

Quantitative ureaplasma studies showed that one of the strains inoculated into two of the chimpanzees was able to multiply and persisted for at least 14 days; the other strain was also shown to persist. Stains of urethral smears showed no evidence of any polymorphonuclear response or increased shedding of urethral epithelial cells accompanying the infection. A seven-day course of neo-terramycin eliminated *U. urealyticum* from the chimpanzees' urethras. *M. Kelsey*

Reference

Taylor-Robinson, D., Csonka, G. W. and Prentice, M. J. (1977). Human intra-urethral inoculation of ureaplasmas. *Quarterly Journal of Medicine*, 46, 109.

Chlamydia pneumoniae (Letter)

W. J. OETGEN (1978).

New England Journal of Medicine, **299**, 48

New *Chlamydia* isolation methods applied to the current medical practice and epidemiology

F. CATALAN, E. EDLINBERGER, A. SIBOULET, S. BURTCHAEILL, AND E. OUIZMAN (1978). *Annales de Microbiologie*, **A129**, 329-340

Cultures of *Chlamydia trachomatis* in mouse peritoneal macrophages: factors affecting organism growth

C. C. KUO (1978).

Infection and Immunity, **20**, 439-511

Effect of ammonium ion concentration and osmotic pressure on growth of *Ureaplasma urealyticum* (T-strain mycoplasma)

I. A. SAYED AND G. E. KENNY (1978).

Journal of Bacteriology, **134**, 967-981

Trichomoniasis

Isolation of *Trichomonas vaginalis* resistant to metronidazole

J. THURNER AND J. G. MEINGASSNER (1978). (Letter) *Lancet*, **2**, 738

Genital herpes

Treatment with levamisole of recurrent herpes genitalis

T.-W. CHANG AND N. FIUMARA (1978).

Antimicrobial Agents and Chemotherapy, **13**, 809-831

A double-blind study was carried out to investigate the possibility of therapeutic effect of levamisole on recurrent pro-genital herpes. One hundred and nine patients, including 53 women, entered the study, but only 75 completed. Levamisole, 50 mg three times daily for three days, was started at the first sign of recurrence. The study period consisted of six visits or 12 months, whichever came first. No statistical differences were observed between levamisole and placebo groups when comparing the duration of the lesion and the degree of pain, although less pain was observed among those on levamisole. The interval between attacks was increasingly prolonged in the levamisole-treated group and reached a significant level at the sixth visit. However, analysis on the basis of mean

cumulative number of days between attacks showed no significant differences throughout the study period. Because of occasional neutropenia and generalised urticaria, and because of the absence of clear-cut clinical improvement of statistical significance, levamisole was considered of limited benefit to patients with recurrent genital herpes infection.

Authors' summary

Ineffectiveness of topical ether for the treatment of genital herpes simplex virus infection

L. COREY, W. C. REEVES, W. T. CHIANG, L. A. VONTVER, M. REMINGTON, C. WINTER, AND K. K. HOLMES (1978).

New England Journal of Medicine, **299**, 237-239

Herpesvirus simplex is ether sensitive, and because of this the application of diethyl ether is often recommended for the therapy of genital herpetic infection. In this study from Seattle the efficacy of this treatment was evaluated; a double-blind trial against a placebo was not possible because of the obvious smell of ether, so an open plan was adopted.

The study group comprised 71 patients (37 men and 34 women) experiencing 81 episodes of culture-proved genital herpetic infection. Of 35 patients with primary genital infections with herpesvirus simplex 17 received treatment with ether applied four times a day for seven days, and 18 no therapy. Of 46 episodes of recurrent infection, 26 were treated with topical ether and 20 with no therapy.

All patients experienced burning pain on the application of ether, and many commented that this was 'worse than the disease'. Topical ether did not shorten the duration of either primary or recurrent genital herpetic infection and did not prevent or delay recurrent episodes.

J. D. Oriel

Antibodies to herpes simplex virus type 2 in husbands of patients with cervical carcinoma (Letter)

M. KUNKEL, H. OBERENDER, L. GARTNER, R. HOFMANN, K.-H. RÄTZ, AND H. HEISE (1978).

Lancet, **2**, 585

Relation of herpesvirus hominis type II to carcinoma of the cervix: an animal model for the induction of long-term latency of herpesvirus hominis type II

S. M. TOBIN, W. D. WILSON, E. N. FISH, AND F. R. PAPAIN (1978).

Obstetrics and Gynaecology, **51**, 707-712

Other sexually transmitted diseases

Antimicrobial susceptibility of *Haemophilus ducreyi*

G. W. HAMMOND, C. J. LIAN, J. C. WILT, AND A. R. RONALD (1978).

Antimicrobial Agents and Chemotherapy, **13**, 608-612

The susceptibility of 19 isolates of *Haemophilus ducreyi* from a recent chancroid outbreak and four reference strains was determined *in vitro* to 13 antimicrobial agents. The rabbit intra-dermal test for virulence was positive for all of the local isolates, but not for the reference strains. The 'nonvirulent' reference strains were inhibited by lower minimum inhibitory concentrations (MICs) of most agents tested. For the virulent isolates, the range of MICs (in micrograms per millilitre) of the following were: of vancomycin, 8 to 128; of polymyxin, 32 to 128; of cloxacillin, 32 to 64; of tetracycline, 0.5 to 32; of cephalothin, 4 to 8; of doxycycline 0.25 to 8; and of kanamycin, 1 to 8. Three strains were resistant to penicillin and ampicillin (MIC > 128 µg/ml), and these three strains produced β-lactamase. The remainder were susceptible to 4 µg/ml. All strains were susceptible to rifampicin (MIC ≤ 1 µg/ml), chloramphenicol (MIC ≤ 4 µg/ml), sulfisoxazole (MIC ≤ 8 µg/ml), and nalidixic acid (MIC ≤ 8 µg/ml). These susceptibilities of *H. ducreyi* indicate several antimicrobial agents that may be effective for chancroid treatment and support the use of vancomycin in a selective medium for the culture of chancroid genital ulcers.

Authors' summary

Determination of the hemin requirement of *Haemophilus ducreyi*: evaluation of the porphyrin test and media used in the satellite growth test

G. W. HAMMOND, C.-J. LIAN, J. C. WILT, W. L. ALBRITTON, AND A. E. RONALD (1978). *Journal of Clinical Microbiology*, **7**, 243-246

Miscellaneous

Non-specific vaginitis. Role of *Haemophilus vaginalis* and treatment with metronidazole

T. A. PHEIFER, P. S. FORSYTH,
M. A. DURFEE, H. M. POLLOCK, AND
K. K. HOLMES (1978).

New England Journal of Medicine, **298**,
1429-1434

To assess the cause of non-specific vaginitis, we performed a prospective case-control study of vaginal flora and a randomised unblinded trial of different therapies. *Haemophilus vaginalis* was isolated from 17 of 18 women with signs of vaginitis but only one of 18 normal matched controls ($P < 0.002$). The concentration of anaerobic bacteria in vaginal washings also was increased in patients. Clinical improvement and eradication of *H. vaginalis* occurred in one of seven patients given sulphonamide vaginal cream, two of 15 given oral doxycycline, nine of 27 given oral ampicillin, and 80 of 81 given oral metronidazole. On the seventh day of therapy signs of non-specific vaginitis persisted in 31 of 31 with, and in two of 92 without, persistent *H. vaginalis* infection ($P < 0.001$). These data suggest the causal role of *H. vaginalis* in non-specific vaginitis, possibly in concert with vaginal anaerobes.

The widespread use of sulphonamide creams is inappropriate. Metronidazole is effective, but its efficacy must be weighed against its possible toxicity.

Authors' summary

Anogenital infection with *Neisseria meningitidis* in homosexual men

F. N. JUDSON, J. M. EHRET, AND

T. C. EICKHOFF (1978).

Journal of Infectious Diseases, **137**,
458-463

Among homosexual men anal infection with *Neisseria meningitidis* was more prevalent (15 of 731 men) than expected and significantly more prevalent than urethral infection with *N. meningitidis* (three of 669 men, $P < 0.01$). Anal infection was also significantly more prevalent among homosexual men than among heterosexual women (two of 1197 women, $P < 0.001$). These differences in rates of prevalence may be best explained by a preference of meningococci for anal mucosa and by the common homosexual practice of oro-anal sexual contact. Serogrouping of the 17 anal and three

urethral isolates showed a broad representation of serogroups often found in meningococcal pharyngeal carriage in the community. Of 14 patients who returned for a test-of-cure culture within seven days of treatment with an antibiotic regimen recommended for anogenital gonococcal infection, each was culture-negative for *N. meningitidis*. Minor anal symptoms in three men and profuse urethral discharges in two men resolved with treatment.

Authors' summary

***Haemophilus vaginalis* in non-specific vaginitis (Leading article)**

Lancet, 1978, **2**, 459-460

Penetration of antibiotics into cerebrospinal fluid and brain tissue

R. W. A. BARLING AND J. B. SALKON (1978).

Journal of Antimicrobial Chemotherapy,
4, 203-229

Sexual assault: the victim's psychology and related problems

O. KNOPF (1978).

Mount Sinai Journal of Medicine, **45**,
1-13

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Contents

An assessment of the Microcult-GC culture test

P. F. UNSWORTH, H. TALSANIA, AND I. PHILLIPS (Gt Britain) page 1

Serum immunoglobulin response in uncomplicated gonorrhoea

A. McMILLAN, GILLIAN McNEILLAGE, H. YOUNG, AND SHEILA R. BAIN (Gt Britain) page 5

Diagnosis of gonorrhoea in women

R. N. THIN, AND ELIZABETH J. SHAW (Gt Britain) page 10

Pharyngeal colonisation by *Neisseria gonorrhoeae* and *Neisseria meningitidis* in black and white patients attending a venereal disease clinic

R. C. NOBLE, R. M. COOPER, AND BETTY R. MILLER (USA) page 14

Inhibition of *Neisseria gonorrhoeae* by normal human saliva

A. MELLERSH, A. CLARK, AND S. HAFIZ (Gt Britain) page 20

Short-term treatment of gonorrhoea with intramuscular and oral forms of trimethoprim-sulphamethoxazole

A. LASSUS AND O-V. RENKONEN (Finland) page 24

Antibodies to *Chlamydia trachomatis* in acute salpingitis

J. D. TREHARNE, K. T. RIPA, P-A. MÅRDH, L. SVENSSON, L. WESTRÖM, AND S. DAROUGAR (Gt Britain and Sweden) page 26

Ureaplasma urealyticum and *Mycoplasma hominis* in chlamydial and non-chlamydial nongonococcal urethritis

D. TAYLOR-ROBINSON, R. T. EVANS, E. D. COUFALIK, M. J. PRENTICE, P. E. MUNDAY, G. W. CSONKA, AND J. K. OATES (Gt Britain) page 30

Treatment of nongonococcal urethritis with rifampicin as a means of defining the role of *Ureaplasma urealyticum*

E. D. COUFALIK, D. TAYLOR-ROBINSON, AND G. W. CSONKA (Gt Britain) page 36

Herpesvirus hominis antibodies among children and young adults in Ibadan

A. O. SOGBETUN, D. MONTEFIORE, AND C. N. ANONG (Nigeria) page 44

Necrotising balanitis due to a generalised primary infection with herpes simplex virus type 2

J. F. PEUTHERER, ISABEL W. SMITH, AND D. H. H. ROBERTSON (Gt Britain) page 48

Current approaches to the diagnosis, treatment, and reporting of trichomoniasis and candidosis

B. H. O'CONNOR AND M. W. ADLER (Gt Britain) page 52

A theory of the epidemiology of gonorrhoea

W. F. FELTON (Gt Britain) page 58

Treatment failure with penicillin in early syphilis

A. J. H. GILES AND A. G. LAWRENCE (Gt Britain) page 62

Correspondence page 65

Notice page 67

Report of the Medical Society for the Study of Venereal Diseases page 68

Abstracts page 70

ASTM CODEN: BJVDK 55 (1) 1-74 (1979)

PUBLISHED BY BRITISH MEDICAL ASSOCIATION, TAVISTOCK SQUARE, LONDON WC1H 9JR, AND
PRINTED IN ENGLAND BY DAWSON & GOODALL LTD., THE MENDIP PRESS, BATH

ISSN 0007-1226

April 1979 Vol. 55 No. 2

REC.
16.10.79

British Journal of Venereal Diseases

BRITISH MEDICAL ASSOCIATION TAVISTOCK SQUARE LONDON WC1

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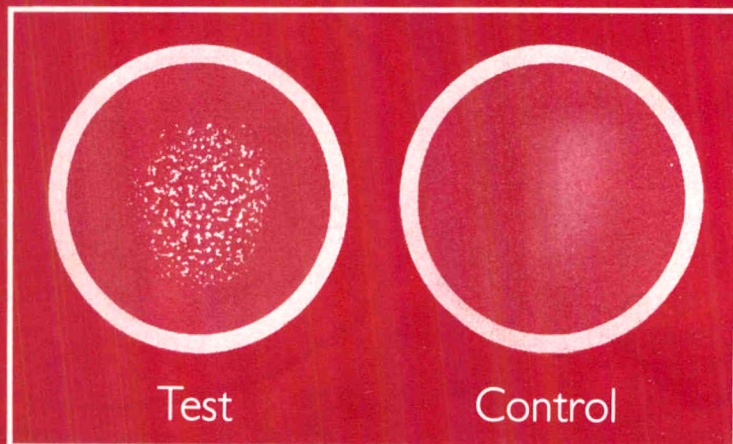
Reference 1 Balmer, J.A. (1976) Am J Obstet Gynaec 126: 4436-441

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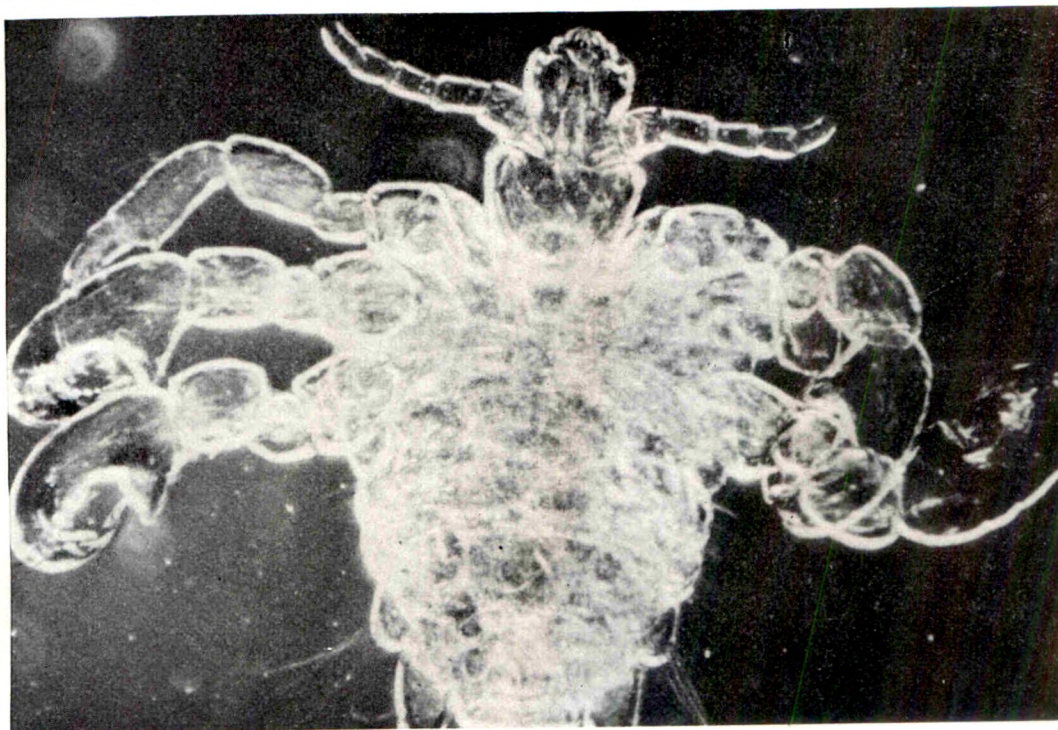
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- 1 Brit. J. Vener. Dis. (1975), 51, 97-98
 - 2 Brit. J. Vener. Dis. (1975), 51, 206-209
 - 3 Brit. J. Vener. Dis. (1976), 52, 43-45
 - 4 Brit. J. Vener. Dis. (1978), 54, 107-111
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Bulletin of the International Union against the Venereal Diseases and Treponematoses

29th General Assembly, Leeds, 21-25 May, 1978

Registration of the delegates took place at the Dragonara Hotel, Leeds, from 12 noon on Sunday, 21 May. One hundred and sixty-nine delegates registered, 48 of them bringing their wives or husbands. A further 30 or so delegates attended the meeting without registering, which brought the total number of delegates to the Assembly to approximately 200, coming from 25 different countries in all parts of the world. The Assembly immediately followed the two-day spring meeting of the Medical Society for the Study of Venereal Diseases in which many IUVDT delegates had taken part.

A meeting of the Executive Council of the IUVDT was held on the afternoon of 21 May and delegates were entertained to a cocktail party in the evening. On the morning of 22 May 12 papers were read on 'Recent advances in microbiology and immunology of gonorrhoea with their practical application including treatment' and in the afternoon a further 11 papers were read on 'Recent advances in the immunology of syphilis'. On 23 May 16 papers were read in the morning and early afternoon on 'Recent advances in the study of STD due to viral and other agents'. In the late afternoon and on the following morning 21 papers were read on 'New trends in the epidemiology of STD'. A detailed programme of the scientific sessions was as follows:

SESSION 1: Theme—Recent advances in microbiology and immunology of gonorrhoea with their practical application including treatment

Theme co-ordinator: Dr Paul Wiesner (USA); Chairmen: Dr A. Reyn (Denmark) and Dr Stuart Brown (USA)

Sensitivity pattern of *Neisseria gonorrhoeae* to penicillin and screening for β -lactamase production in Ibadan: A. O. Sogbetun and A. O. Osoba (Nigeria).

Surveillance of β -lactamase-producing *N. gonorrhoeae* in Africa: P. Piot, M. Duchassin, A. Meheus, Squires C. Bosmans, T. L. Muyembe, M. C. Mupapa, and E. Van Dyck (Belgium).

Antibody responses in gonorrhoea: A. A. Glynn, C. Ison (UK).

Mucosal antibodies in uncomplicated gonococcal infections: A. McMillan (UK).

Penicillin sensitivity of *N. gonorrhoeae* in West Germany: D. Petzoldt, K. Grunder, U. Neubert.

Effect of different preparation procedures on the ultrastructure of gonococci: W. Gebhart, W. Jurecka, J. Söltz-Szöts, J. Thurner, A. Ellinger, and K. Kitz (Austria).

Gonococcal transformations *in vivo*: P. Novotny (UK).

Frequency of urogenital infections of mixed aetiology: gonorrhoea and other infections: A. Siboulet and F. Catalan (France).

Barefoot bacteriological diagnosis of gonorrhoea in areas with limited medical facilities: G. A. Olsen (Denmark).

Spontaneous disappearance of *N. gonorrhoeae* in the tonsillar pharyngeal region: J. Wallin (Sweden), read by Dr Stuart Brown (USA).

Treatment of uncomplicated gonorrhoea in men with single doses of 2.5 g thiamphenicol (Glitisol): C. Santos (Brazil).

Preservation of the gonococcus by gelatin-disc method: S. Yamai, Y. Obara, T. Nikkawa, Y. Shimoda, and Y. Miyamoto (Japan).

SESSION 2: Theme—Recent advances in the immunology of syphilis

Theme co-ordinator: Dr James N. Miller (USA)

Role of humoral versus cellular mechanisms of resistance in the pathogenesis of human syphilis: M. Metzger (Poland).

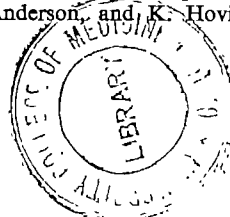
Differential lesion development in rabbits from single and graded doses of *Treponema pallidum*: N. H. Bishop (USA).

Changes following treatment of *Borrelia duttoni* infection in mice and its relationship with the Jarisch-Herxheimer reaction: D. J. M. Wright (UK).

Comparison of several cardiolipin and treponemal tests in the serodiagnosis of yaws: H. E. Menke, J. Veldkamp, and E. Stolz, E. A. Brunings, P. L. A. Niemel, and A. Notowicz (Holland).

Attenuated yaws in central north Surinam: P. L. A. Niemel, E. A. Brunings, and H. E. Menke (Surinam).

Comparison of the ultrastructure of immunoimmobilised *T. pallidum* and treponemes present in syphilitic patients: H. A. Nielsen, A. Burch-Anderson, and K. Hovind-Hougen (Denmark).



Recent developments in the serum diagnosis of syphilis: A. Luger (Austria).

Standardisation programme for diagnostic products and tests for syphilis: B. McGrew (USA).

Scheme of IgM and IgG in human venereal syphilis and in experimental treponematoses: P. Hamplén (France). Evaluation of a double-staining technique for the fluorescent treponemal antibody absorption (FTA-ABS) test: E. F. Hunter, R. M. McKinney, E. Maddison, D. D. Cruce (USA).

The role of cellular hypersensitivity to *Treponema pallidum* in human syphilis: P. F. Friedman (UK).

SESSION 3: Theme—Recent advances in the study of STD due to viral and other agents

Theme co-ordinator: Dr R. D. Catterall (UK)

Late manifestations of lymphogranuloma venereum: C. N. Sowmini, and K. N. Gopalan (India).

Seroepidemiological study of lymphogranuloma venereum in West Nigeria: A. O. Osoba (Nigeria).

The biology of *Trichomonas vaginalis*: M. Muller (USA).

Sexually transmitted hepatitis B: F. Lim (UK).

Follow up of patients with hepatitis B surface antigen: P. Simmons (UK).

Group B streptococcal infection: J. Knox (USA).

Herpes simplex—animal model for therapy evaluation: M. Jarratt (USA).

Immunological aspects of herpes genitalis: D. Fanta, and E. M. Kokoschka (Austria).

Vaccination in herpes genitalis: T. Nasemann (West Germany).

Therapeutic aspects of herpes genitalis: J. Söltz-Szöts, D. Fanta (Austria).

Chlamydial genital infection: A. Siboulet, and F. Catalan (France).

Recent advances in serodiagnosis of chlamydial genital infections: J. D. Trehanne (UK).

Chlamydial genital infection in certain countries of the Middle East and Africa: S. Darougar, B. Aramesh, N. R. Jones, J. D. Trehanne, and A. O. Osoba (UK, Iran, and Nigeria).

Prevalence of chlamydiae in genital infections: H. G. Robson (Canada).

A case of trichomonal vaginitis resistant to metronidazole: J. Thurner, and J. G. Meingassner (Austria).

Condylomata acuminata—therapeutic considerations: E. Gupta (UK).

SESSION 4: Theme—New trends in the epidemiology of STD

Theme co-ordinators: Drs G. Causse, T. Guthe, A. Siboulet (Switzerland, Norway, and France)

Syphilis as viewed from the TPHA test: Y. Onoda and N. Okubo (Japan).

Studies on monospecific FTA-ABS: K. Mizuma, Y. Kataniwa, and T. Sato (Japan).

Therapeutic effect of oral doxycycline in syphilis: Y. Onoda (Japan).

Situation of STD in Latin America: C. Santos (Brazil).

An alternative to the problem of insufficient physician manpower in the clinical care of sexually transmitted diseases: C. W. Parton, and S. M. Sgroi (USA).

Whither candidosis?: S. S. Bello (Nigeria).

SESSION 5: Theme (cont.)—New trends in the epidemiology of STD

Critical evaluation of the venereal disease control campaign in Poland: J. Towpik (Poland).

Annual Report 1976—Venereal Disease Control Division, South Australia: D. Jorgensen (Australia).

Epidemiology of syphilis and gonorrhoea in New Zealand: W. M. Platts (New Zealand).

Contact in Europe: A. Siboulet (France).

Priorities in a venereal disease control programme: R. L. Persad (Canada).

Current treatment for STD in England and Wales: M. W. Adler (UK).

Does gonorrhoea surveillance pay?: R. C. Rendtorff and H. Packer (USA).

Screening for STD among women attending gynaecological clinics in developing countries: A. Onifade, and A. O. Osoba (Nigeria).

Results of routine screening for STD in Austria over six-year period: D. Fanta, W. Gebhart, W. Gross, E. M. Kokoschka, J. Söltz-Szöts, and J. Thurner (Austria).

Trends in the epidemiology of STD in Tamil Nadu: C. N. Sowmini (India).

Management of a gonorrhoea epidemic far from Montreal: R. Morrisset, S. Kasatea, and G. Tarjan (Canada).

Morbidity of syphilis and gonorrhoea in the internees of the Tokyo detection and classification camp for juvenile delinquents: M. Ashizawa, K. Ito, and M. Ishino (Japan). Non-specific urethritis in developed and developing countries: R. R. Willcox (UK).

Gonorrhoea in Sudanese patients attending venereal diseases clinic in Khartoum: E. E. Omar (Sudan).

Illness behaviour and STD: R. M. Harrison (UK).

On the evening of Tuesday 23 May a most impressive and generous reception was given in the Leeds Civic Hall by the Lord Mayor of Leeds.

The General Assembly was held in the afternoon of 24 May and was opened by the President, Dr Axel Perdrup (Denmark), who welcomed the delegates and thanked all those who had helped to make it a success, especially Dr M. A. Waugh, the local organiser, and his staff, Dr J. R. W. Harris, the programme secretary, and the Secretary General, Dr James Jefferiss. The Secretary General then gave his report, saying that he had apologies from Dr Causse of WHO, Professor Canaperia, Professor Hermanns, Dr Vejjabul, and Dr Rajan:

The chief occupation of the Secretary General since our 28th General Assembly in Malta has been the arranging and cancelling of the 29th General Assembly first in

Mexico City then in Acapulco, and arranging it finally here in Leeds. The Assembly was first to have followed the International Dermatological Congress in Mexico City but was cancelled as it was thought by the Executive Committee that more delegates would be attracted to come to the famous resort of Acapulco after a week in Mexico City. Unfortunately, the arrangements had to be cancelled there too because the hotel where we were to have held the Assembly needed a substantial sum of money deposited five months before the arranged dates. By that time only 18 delegates had paid their registration fee, and we could not risk expending the large sum requested without the assurance that enough delegates would attend so that it would be repaid. However, we have finally got together here.

We had 1000 provisional programmes printed for both Acapulco and Leeds, and we are very grateful to the MSSVD who kindly had over 500 of them sent to their members with their other circulars. The remainder were sent out by the Secretary General.

The Executive Committee met in Vienna from 18 to 20 September 1976. The President called our attention to the grievous loss we had sustained by the death of one of our most active members, Dr Bruce Webster of New York. Various routine matters were then attended to, and Dr Siboulet reported on his work in Europe and South America, suggesting that we should form a Latin American regional office separate from the North American region.

We are very glad to welcome the Australian Department of Health as a new member organisation of our Union as well as several other individual members from that and other countries.

The Union was represented by Dr Michael Waugh at the Montreal Symposium on STD in November 1977.

We have continued our close co-operation with the WHO, which as always is most valuable to us, and I am pleased to say that in March this year Dr Mahler wrote on behalf of WHO to inform us that the Executive Board had decided to maintain official relations with our organisation. Since our last meeting we have sent representatives of our Union, including our President, to the 28th World Health Assembly Technical Discussions in April 1975 on 'Social and Health Aspects of Sexually Transmitted Diseases'—the documents and reports of which were circulated to members.

We have sent representatives to meetings of the regional committees of WHO in the USA, Pakistan, Manila, Thailand, Mexico, and India. The symposium on the 'Surveillance and Control of STD', organised by the regional office for Europe of the WHO was held in Vienna from 21 to 25 September, 1976, where we were represented by our President, Professor Axel Perdrup, and Drs Guthe, Siboulet, and Delune, and several others who were also representing their countries. In addition we have been represented at the 30th and 31st World Health Assemblies in May 1977 and May 1978 by our ex-President and Secretary General, Professor G. A. Canaperia. We are very happy to congratulate him on his recent honour from the World Health Organisation of the Leon Bernard Foundation Award in Social Medicine.

The Treasurer, Dr R. D. Catterall (UK) then reported:

I have the honour once again to present the accounts of the International Union against the Venereal Diseases and Treponematoses for the year ended 31 December, 1977. The financial position of the Union is satisfactory and shows further progress since I last reported to you.

During the year under review subscriptions were received from 80 individual members and from 24 different organisations, but, as has so often happened in the past, there were a number of individuals and several organisations that failed to pay their membership fee, despite reminders on more than one occasion.

Nevertheless, income from subscriptions amounted to £1652 and interest on our investments totalled £371, making our total income for the year £2023, compared with £2165 in 1976.

The expenses of the Union amounted to £820 and this includes the sum of £280 which had to be paid towards the abortive arrangements for the Mexican meeting.

The annual subscription of 191 Korunas was once again paid into the Czechoslovakia Obchodni Bank in Prague. Our credit balance at that bank amounts to the equivalent of £365 but remains blocked; this amount is not shown in our accounts.

The balance standing to our account at Coutts Bank, London, at 31 December 1977 was £4828. Total funds of £4761 have been invested in United Kingdom Government stocks, the interest on which contributes usefully to our income and the capital sum forms a solid basis for our finances.

When the expenses of the Leeds meeting have been met in full it is probable that the balance of our accounts will permit further investments to secure our financial position in the future.

One of the difficulties of the Treasurer is to persuade every individual member to pay his subscription and to ensure that all the member organisations pay their annual contributions. I should like to ask all individual members to arrange for a banker's order or other suitable arrangement so that their subscription is paid automatically each year and for members of the Executive Committee and senior members of the Union to ensure that their national organisation pays its subscription every year.

Our accounts are audited annually by Payne, Stone, Fraser and Co., a firm of Chartered Accountants in London.

The report of the Director of the Regional Office for Europe Dr A. Siboulet (France) should then have been read, but owing to his recall home and a misunderstanding by the Secretary General it was not to hand. However, it is now printed as follows:

Since the last meeting of the Executive Committee, we have made concerted efforts to diffuse health information concerning the communicable sexual diseases to the public and to doctors, and made numerous contacts with specialists of widely different countries, and con-

tinued to carry out surveys in order to stipulate the best ways of approaching the public.

Thanks to the constant and efficient help of Dr Coulon, General Director of the Alfred Fournier Institute, we have continued to publish and distribute informative material for doctors and the public at large, and many thanks to Dr Durel, General Secretary, LNFCLPV.

The Regional Office has had numerous visits and has maintained contacts:

- (1) With the WHO and Dr Causse, more especially as a permanent study on gonococcal infections and their clinical, therapeutic, and epidemiological aspects has been under way since 1961.
- (2) With numerous colleagues during the International Symposium on the urogenital trichomoniasis at Alfred Fournier Institute in 1977 and during 'Le Premier Cours International OMS-MST' at Alfred Fournier Institute in 1977.
- (3) With the French Health Ministry, who asked us for details on the dangers of sexually transmitted diseases for tourists in 1977 and 1978, and with Professor Perea and Dr Rodrigues (Spain), Professor Delune (Belgium), Dr Stolz (Holland), Dr Franceschini (Italy), Dr Paramanda (Germany), Professor Brault, Professor Morrisset, Dr Tarjan (Canada), Dr Mobarak (Lebanon), and Professor Diop-Mar (Senegal).
- (4) With Professor Santos de Goiana (Brazil), President of the new union, Latino-Americana contra las Enfermedades Venereas (Union), who visited the regional office in 1977; Professor José Martins Barros is scientific consultant to this Union.

Concerning the Franco-Swedish sectional pilot study, Dr Martin Bouyer has made known the latest results of this study. It was shown that the probable number of patients suffering from STD was approximately 5000 per year, that is 96 times more than the number of patients declared officially.

With the Comité d'Education Sanitaire et Sociale de la Pharmacie Française et la Ligue Nationale Française contre les Maladies Vénériennes, we have published a pamphlet called 'Il n'y a pas de Maladies Honteuses'.

Under the statutes of the Union, sanitary information must be one of our essential goals and we propose to intensify this effort in the years to come.

In conclusion, the present increase of communicable diseases in Europe and other countries incites us to try to find and take new measures in the fight against these diseases; more than ever a close collaboration between the different regional offices is necessary in order to try to reach the public more and to give doctors precise information, which would be brought up to date each year.

There was no report from the Director General of the Regional Office for the Americas but the report of the Regional Director of the South East Asia and Western Pacific Region was given by Dr C. N. Sowmini (India) in the absence of Dr P. Vejjabul. She reported on the many activities of the Region and the highly successful Regional Conference on

Sexually Transmitted Diseases, held in Singapore in January 1977. The latter was commented on very favourably by Dr R. D. Catterall, who represented the Union on that occasion.

With regard to the Council of Europe Dr Guthe reported that nothing significant had taken place since our last meeting.

Dr M. R. Radovanovic, European Regional Officer for Communicable Diseases of the World Health Organisation, then addressed the Union on behalf of the World Health Organisation. He conveyed greetings to us from the WHO and thanks for inviting him to address us. He apologised for the absence of Dr G. Causse, who was attending the WHO General Assembly at Geneva, where several important matters concerning STD were being discussed. He hoped that we would collaborate with WHO even more than in the past, and especially that our regional offices would collaborate with the widely distributed WHO regional offices in the same parts of the world. He suggested that the IUVDT Regional Office for Europe and the WHO European Regional Office should be in especially close collaboration. He gave as an example the great success of the co-operation of our Executive Committee meeting and the European Regional Office Symposium on the 'Surveillance and Control of STD' at Vienna in September 1977. He suggested that the IUVDT in future should try to arrange their meetings so that they would be continuous with a WHO meeting.

The Secretary General then read the recommendations made by the Executive Committee at their meeting on 21 May 1978 for ratification or otherwise by this 29th General Assembly:

- (1a) The formation of a Latin American Region, comprising all countries in the American continent from and including Mexico southwards.
- (1b) The formation of a North-American Region comprising USA and Canada.
- (1c) That all support possible should be given by this Union to Dr A. O. Osoba (Nigeria) in forming an African Region.
- (2) Appointment of officers: President, Dr R. D. Catterall (UK); Secretary General, Dr James Jefferiss (UK) for a further and final year and then Professor Anton Luger (Austria) to take over the office; Treasurer, Dr D. Petzoldt.
- (3) Vice Presidents: Dr Pierre Durel; Dr Hubert Delune; Dr Norton Brandao; Dr Thorstein Guthe.
- (4) Regional Directors: Europe, Dr André Siboulet; North America, Professor John Knox; Latin America, Professor Corintha Santos; South-East Asia and Western Pacific, Dr P. Vejjabul.

- (5) Technical Members of the Council: Dr P. G. Bakker (Netherlands); Dr C. N. Sowmini (India); Professor J. Towpik (Poland); Professor L. Juhlin (Sweden); Professor G. Elste (East Germany); Dr V. S. Rajan (Singapore); Dr J. P. Brault (Canada); Dr L. Nicholas (USA); Dr J. R. W. Harris (UK); Dr A. O. Osoba (Nigeria); Dr D. Jorgensen (Australia); Dr G. Coulon (France); and Dr Y. Onoda (Japan).
- (6) That the title 'Permanent Consultants' should be dropped as no place for them appears in the Union's statutes and that the present holders of this title should be known as 'Honorary Members' and that in future those who according to Article 5 in the Statutes have 'Rendered important services to the Union' should be classed as Honorary Members also.
Present Honorary Members and those now proposed: Dr Ambrose King; Dr E. Hermanns; Dr H. Brun-Pedersen; Professor F. Foldvari; Professor G. A. Canaperia; Dr C. S. Nicol; Mrs Josephine V. Tuller; Dr M. Tottie; and Professor Axel Perdrup.
- (7) Legal Counsellor: Mr P. Pfeiffer.
- (8) Place and date of 30th General Assembly: East Berlin, last week in May 1980, at the invitation of Dr G. Elste (DDR). Themes for the General Assembly to be discussed and finally decided on at Copenhagen 15-16 June 1979. The registration fee for the 30th General Assembly would be \$50.

All these recommendations were agreed to unanimously. Under 'any other business' Dr Verling (Eire) invited the Union to hold a future General Assembly in Dublin and Professor Axel Perdrup brought up the matter of the great difficulty in obtaining penicillin (PAM), which is now only made by one manufacturer in the United Kingdom

for export only as its production is uneconomic. The difficulty in obtaining procaine penicillin is almost as great. It had been hoped that benzathine penicillin would take the place of PAM but recent work has suggested that it is not absorbed into the cerebrospinal fluid. Members were asked to urge their own governments to arrange for a supply and Dr Radovanovic was asked to ask the WHO for its help.

Following the General Assembly the Executive Council met again, and among other matters arrangements for the next Executive Council meeting in Copenhagen and the 30th General Assembly in East Berlin were discussed. It was also decided that the practicalities of circulating a news letter should be explored and that if possible a list of members and their addresses should be circulated annually. In view of the very high cost the Union could no longer afford to have simultaneous translation at their Assemblies. It was suggested that all those submitting papers should supply a short summary in French or English, as the case might be, which would be read out to the Assembly.

A very successful banquet was held in the evening, at which Dr T. Guthe spoke warmly of the retiring President Professor Axel Perdrup and thanked him for all the good work he had done for the Union during his term of office. Professor Perdrup replied and thanked all those responsible for the success of this General Assembly and in particular Dr Waugh, the local organiser, to whom he presented a copy of an eighteenth century manuscript on gonorrhoea in view of his interest in the history of medicine.

The final event was a visit to the ancient City of York on the next day, Thursday, 25 May.

Theme 1: Recent advances in the microbiology and immunology of gonorrhoea

Sensitivity of *Neisseria gonorrhoeae* to penicillin in West Germany

D. PETZOLDT, K. GRÜNDER, AND U. NEUBERT

From the University Medical School, Lübeck; and the Department of Dermatology, University of Munich, West Germany

SUMMARY The sensitivity of gonococci to penicillin in Germany has largely decreased in the last 22 years. No significant decrease has occurred, however, in the last three years, but the results show that a slight tendency to decrease may be persisting.

Introduction

Information on the sensitivity of *Neisseria gonorrhoeae* to penicillin in Germany is meagre, but sensitivity seems to be decreasing.

In Hamburg in 1955 Meyer-Rohn (1970) stated that the minimum inhibitory concentrations (MICs) did not exceed 0.001 IU/ml. In 1961 the range of MICs was 0.05-0.18 IU/ml. In 1969 no further decrease was established.

In Munich the sensitivity of *N. gonorrhoeae* to penicillin was determined in 1962 and in 1974. In 1962 more than 90% of 250 strains were of normal sensitivity (IC_{50} of 0.063 IU/ml or lower) (Röckl, 1972), whereas in 1974 only 56% of 104 strains were sensitive (Petzoldt and Reyn, 1976). In addition, the peak of the distribution curve within the sensitive range had moved to the right (Fig. 1).

In Lübeck the penicillin sensitivity of gonococci was determined in 1976. Seventy-seven per cent of the strains tested were found to be sensitive (Gründer and Petzoldt, 1977) (Fig. 2).

The aim of the present study is to investigate the strains collected in 1977 and to compare them with the results quoted above.

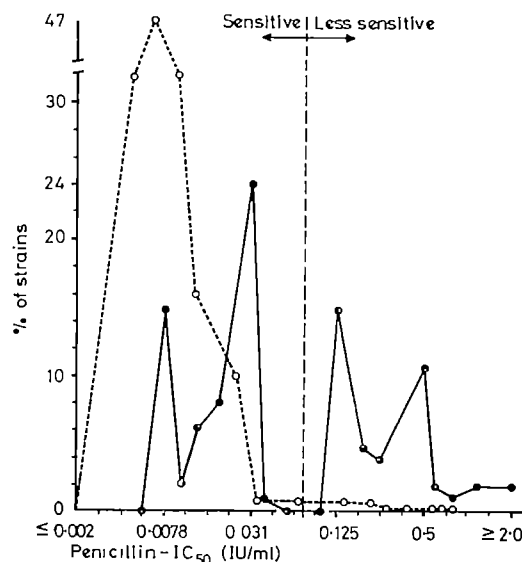


Fig. 1 Penicillin sensitivity of *Neisseria gonorrhoeae* in Munich (1962 and 1974)

○—○ 1962; ●—● 1974

Present study

One hundred and fifty-four strains of gonococci (77 from Munich and 77 from Lübeck) were stored in liquid nitrogen. The sensitivity testing was performed

Address for reprints: D. Petzoldt, Abteilung für Dermatologie, Klinikum der Medizinische Hochschule Lübeck, Ratzeburger Alle 60, D-2400 Lübeck, West Germany

Received for publication 15 June 1978

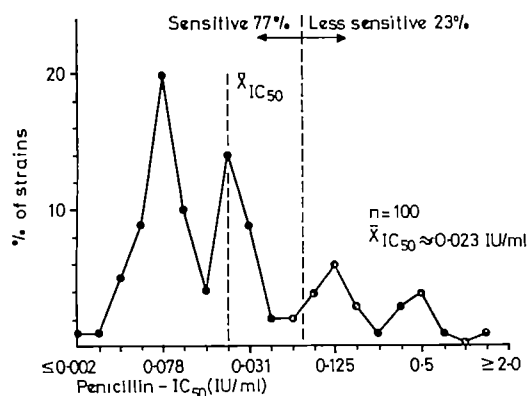


Fig. 2 Penicillin sensitivity of *Neisseria gonorrhoeae* in Lübeck (1976)

by the plate dilution method of the World Health Organisation Gonococcus Reference Centre, Copenhagen (Reyn *et al.*, 1963).

Results and discussion

In Munich 52% of the strains showed normal sensitivity. The average IC_{50} was 0.068 IU/ml (Fig. 3). The results in 1974 were very similar.

In Lübeck 69% of the strains showed normal sensitivity; in comparison with the results of 1976 (77%) this means a definite decrease in sensitivity. The mean IC_{50} was 0.032 IU/ml in 1977 and 0.023 IU/ml in 1976 (Fig. 4). There is evidently no significant difference between the two results.

A comparison of the sensitivity of the strains isolated in Munich with those isolated in Lübeck is of interest. The strains from Munich had a mean

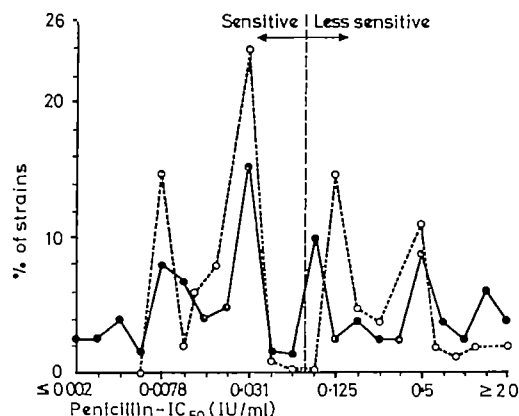


Fig. 3 Penicillin sensitivity of *Neisseria gonorrhoeae* in Munich (1974 and 1977)
○—○ 1974; ●—● 1977

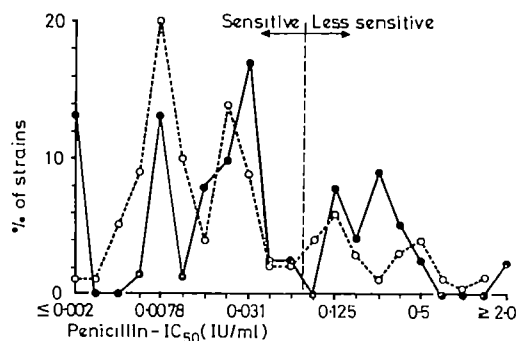


Fig. 4 Penicillin sensitivity of *Neisseria gonorrhoeae* in Lübeck (1976 and 1977)
○—○ 1976; ●—● 1977

IC_{50} of 0.068 IU/ml and were significantly less sensitive than the strains from Lübeck with an average IC_{50} of 0.032 IU/ml ($t=2429$, $P<0.02$).

The cause of this difference can only be a matter of speculation. Undoubtedly, the extent of clandestine prostitution is greater and the supervision of the prostitutes by the medical services is more difficult in a big capital city like Munich than in Lübeck, a city of only 240 000 inhabitants. The more pronounced decrease in sensitivity to penicillin of strains in Munich may, therefore, reflect a greater illicit administration of this antibiotic.

In February 1978 a penicillinase-producing strain was isolated in Munich (Neubert and Ruckdeschel, 1979). A 33-year-old German patient, who had had sexual intercourse with a prostitute in Cebu (Philippines), complained of a purulent discharge. *N. gonorrhoeae* was isolated, but treatment with penicillin was unsuccessful. A single injection of 1.5 g cefuroxime and 1 g probenecid orally eradicated the symptoms within a few hours. Control investigations gave negative results. The production of β -lactamase was demonstrated with a chromogenic cephalosporin (Glaxo 87/132). The pattern of susceptibility to various antibiotics is shown in the Table (Neubert and Ruckdeschel, 1979).

Table Minimum inhibitory concentrations (μ g/ml) of the isolated β -lactamase-producing strain (Munich 966/78)

Antibiotic	Inoculum size (organisms/ml)			Result
	10^5	10^6	10^7	
Benzylpenicillin	16	64	256	Resistant
Cefuroxime	0.25	0.25	0.25	Sensitive
Spectinomycin	16	16	16	Sensitive
Tetracycline	2	4	4	Moderately sensitive

The strain also had diminished susceptibility to tetracycline; this corresponds to the characteristics of other strains of Far East origin (Percival *et al.*, 1976).

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Effect of different preparation procedures on the ultrastructure of gonococci

W. GEBHART,* W. JURECKA,* J. SÖLTZ-SZÖTS,* J. THURNER,* A. ELLINGER,† AND K. KITZ†

From the *Epidemiology Unit of the Department of Dermatology II; and the †Institute for Micromorphology and Electron Microscopy, University of Vienna, Austria‡

SUMMARY Culture samples of *Neisseria gonorrhoeae* and *Escherichia coli* were studied by transmission and electron microscopy to evaluate the effect of different preparation procedures on the piliation of these organisms. Unfixed as well as fixed bacteria showed only few, long, filamentous appendages when investigated in ultrathin sections, negatively stained specimens, or critical-point dried preparations. Snap-frozen specimens of *E. coli* showed many short and thin pili after being shadowed with carbon and platinum whereas those of *N. gonorrhoeae* showed only some type-C-like pili. Thus, the number and morphological appearance of pili appear to be greatly influenced by the preparation techniques used for study by electron microscopy. Conclusions as to the type and the infectivity of a bacterial strain can, therefore, not be based on purely morphological criteria.

Introduction

The physical, chemical, and adhesive properties of pili in *Enterobacteriaceae* have been well documented in the past (Brinton, 1965; Duguid *et al.*, 1966, 1969). Consequently, it has been recognised that pili or morphologically similar structures occur in a wide range of Gram-negative organisms.

Many investigators have studied the surface of *Neisseria gonorrhoeae* in recent years. The presence of pili on Kellog type 1 and type 2 gonococci as well as their absence on other non-virulent types (Jephcott *et al.*, 1971; Swanson *et al.*, 1971) have stimulated discussion and theories. Recently, Haustein and Uehrlings (1976) suggested that non-piliated gonococci were important epidemiologically, since they had found non-piliated organisms in 20% of asymptomatic cases of gonorrhoea. The negative-staining technique had been used in these investigations. Using the same technique, however, we have seen differences in the number and in the appearance of pili—even in samples from the same culture—depending on the dye applied for the negative-staining.

We have therefore carried out a controlled study on gonococcal pili using different preparation procedures but following the same two-day culture techniques as Haustein and Uehrlings (1976) had used in their study.

Material and methods

Two-day-old cultures of gonococci cultured on Thayer-Martin agar and one-day-old cultures of *Escherichia coli* cultured on Bouillon agar were used for this study. For preparations of the bacteria the following techniques were used simultaneously:

- (1) Small pieces of agar with bacterial colonies were fixed with buffered glutaraldehyde and osmium tetroxide (OsO_4), dehydrated with alcohol, and embedded in Epon 812.
- (2) Gonococci and coliform bacilli grown on culture plates were placed in glutaraldehyde with a platinum loop, gently centrifuged, postfixed with OsO_4 , and embedded in Epon 812. Ultrathin sections of cultures by both methods were stained with uranyl acetate and lead citrate and examined in a Philips EM 200 electronmicroscope.
- (3) Glutaraldehyde-fixed and unfixed bacteria were placed on Formvar-coated electron-microscope (EM) grids and stained with uranyl acetate or phosphotungstic acid (PTA). To place the bacteria on the EM grids two

‡In co-operation with the Ludwig Boltzman Institute for Research on Infectious Venero-dermatological Diseases.

Address for reprints: Professor J. Söltz-Szöts, Allgemeines Krankenhaus des Stadt Wien, II Universitäts-Hautklinik, 9 Alserstrasse 4, 4-1090 Vienna, Austria

Received for publication 16 November 1978

different techniques were used: (a) bacteria were suspended in water, Ringer solution, or glutaraldehyde and a drop of these suspensions was allowed to dry on the grids; and (b) the coated EM grids were directly put on the bacteria growing on the culture plate and after being dried, stained, and carefully washed in distilled water the preparations were examined in a Zeiss EM 9 electron-microscope.

- (4) For scanning electronmicroscopy the critical-point drying method described by Fromme *et al.* (1972) was used. Bacteria were fixed by suspension in glutaraldehyde. After filtration of this solution through a millipore filter the bacteria remaining on the filter were dehydrated with alcohol and transferred in Freon 11 and then in Freon 13 (pressure 39.36 mm Hg, temperature 28.8°C). After drying and decompression the bacteria were sprayed with carbon and gold and examined in a Cambridge scanning electronmicroscope.
- (5) EM grids with unfixed bacteria on the Formvar film were frozen in liquid nitrogen. These preparations were dried in a modified Leybold EPA 100 freeze-etching machine at -90°C and a pressure of about 2×10^{-7} millibars. After complete drying the grids were shadowed with a thin layer of carbon and platinum at -110°C and 2×10^{-7}

millibars. An angle of 15° C was chosen for the coating procedure in order to achieve sufficient contrast. Investigation of these specimens was performed in a Philips EM 200 electronmicroscope.

Results

TRANSMISSION ELECTRONMICROSCOPY OF ULTRATHIN SECTIONS

Between vacuolised and autolytical cells and cell membrane fragments well-preserved gonococci or coliforms could be seen. It was very difficult or nearly impossible in our preparations, however, to trace a long and thin, more or less unorientated filament like a pilus in an ultrathin section. Some osmiophilic filamentous structures or fragments of filaments could be seen extending between the cells or originating from their outer surface (Fig. 1a, b).

TRANSMISSION ELECTRONMICROSCOPY OF NEGATIVELY STAINED PREPARATIONS

With negatively stained preparations nearly identical results could be seen after the sections had been stained with uranyl acetate or with phosphotungstic acid. In the preparations of gonococci usually 20%–30% of the cells had long filamentous appendages corresponding to type A pili (Fig. 2a). These pili mainly stood in bundles and had a

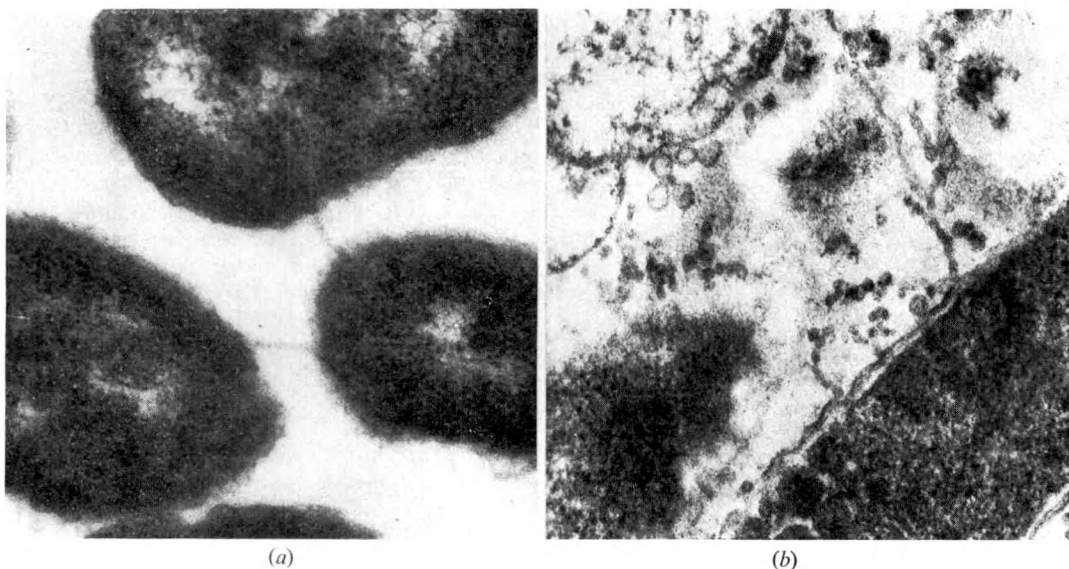


Fig. 1 Transmission electronmicroscopy of ultrathin sections of (a) gonococci and (b) coliform bacilli fixed with glutaraldehyde and osmium tetroxide: (a) some thin filaments are visible extending from the outer surface of one organism to neighbouring bacteria ($\times 65\,000$ magnification); (b) several pearl-string-like fibres originate from the outer cell wall ($\times 52\,000$ magnification)

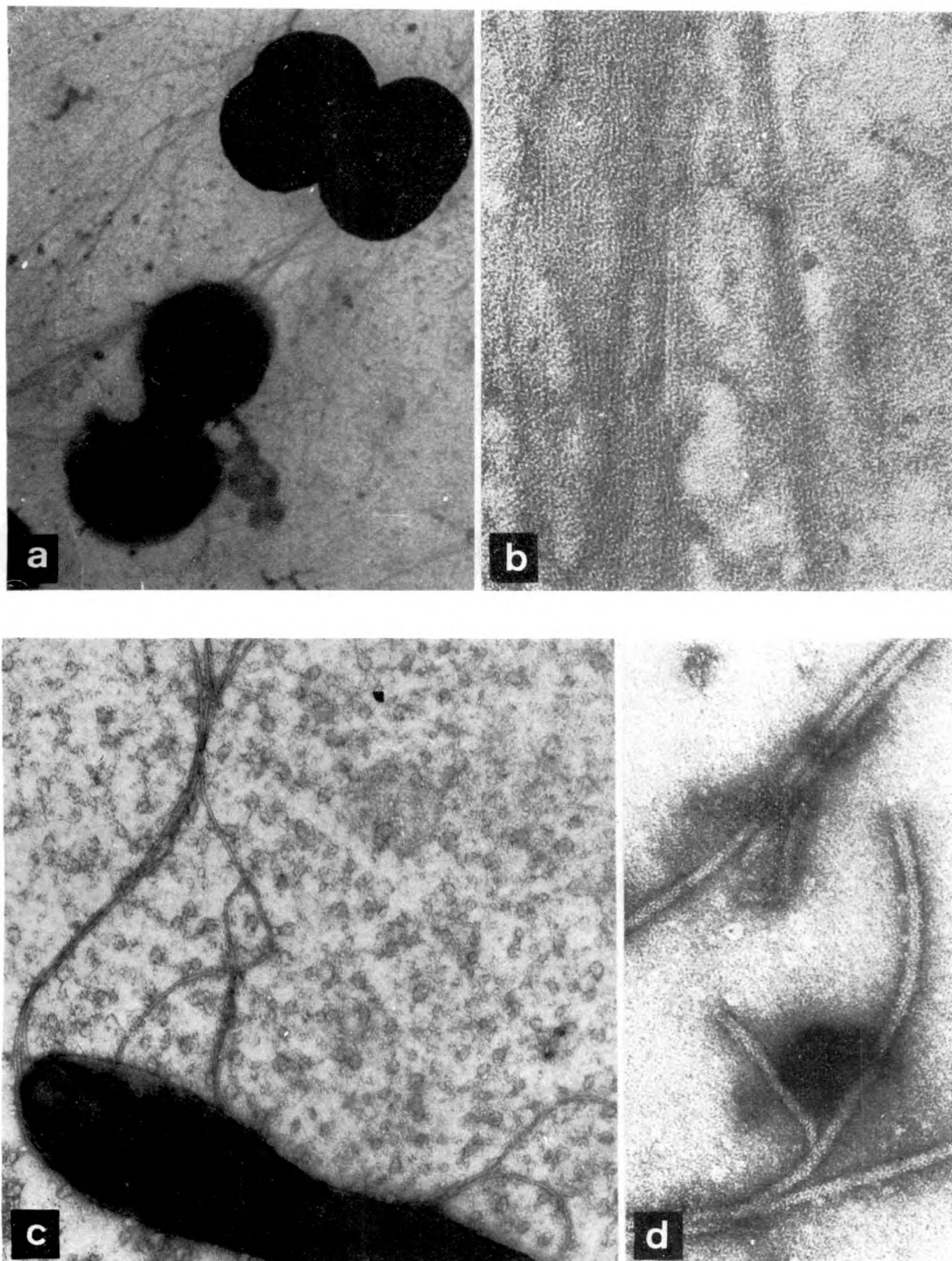


Fig. 2 Unfixed gonococci (a and b) and glutaraldehyde-prefixed coliforms (c and d) negatively stained with PTA: (a) numerous long bundles of thin filaments are present around the organisms ($\times 24\,000$ magnification); (b) a number of parallel membranes arranged in bundles is visible ($\times 100\,000$ magnification); (c) well-defined, long, curved pili are present ($\times 25\,000$ magnification); (d) cross-overs and ramifications of pili are shown ($\times 140\,000$ magnification).

diameter of about 10 nm and a length of several μm . The same preparations frequently also exhibited bundles of parallel membranes along these aggregates of pili, which, because of their thinner diameter, corresponded to structures described as type B pili (Fig. 2b).

Simultaneous preparations from pilated *E. coli* strains also showed very long pili. They were curved and irregularly arranged; at a higher magnification they showed some crossovers (Fig. 2c, d).

SCANNING ELECTRONMICROSCOPY OF FREEZE-DRIED PREPARATIONS

With scanning electronmicroscopy the critical-point dried preparations of gonococci showed no recognisable pili. Only rarely were some thin fibres seen extending from one organism to a neighbouring one (Fig. 3) similar to the structures present in the ultrathin sections.

TRANSMISSION ELECTRONMICROSCOPY OF SHADOWED PREPARATIONS

Finally, in the platinum-carbon shadowed preparations of the samples mentioned above no type A pili could be detected at all (Fig. 4a). Only a few cells showed single long pili with a knob at the end (Fig. 4b). These structures have been described as type C pili (Novotny and Turner, 1975). Most of the gonococci had no recognisable pili, however,

but only a pronounced outer membrane (Fig. 4a). In preparations of *E. coli* more very short and thin pilar structures could be seen all over the surface of the cells (Fig. 5a, b). These were also seen wrapped around the cells and, occasionally, at some distance from the membrane (possibly after having lost their contact with the cell itself).

Discussion

Our investigations into the comparative effects of different preparation procedures on the ultrastructural appearance of gonococci and coliform bacilli gave varied results. Transmission electronmicroscopy of ultrathin sections gave the impression that only occasionally was a pilum tangentially cut, but it was never possible to follow these structures over a longer distance. Thus, this technique itself is not suitable for the evaluation of pilar structures at all. Scanning electronmicroscopy of critical-point dried material was also not satisfactory in our preparations.

Negative-staining mainly produced long bundles of type A pili, after staining of gonococci and coliforms with phosphotungstic acid and uranyl acetate. The same cultures, however, exhibited only type B and C pili or even no pili when examined after freeze-drying and shadowing with platinum.

These discrepancies in the number and morphological appearance of pili indicate strongly that the pilation of both *N. gonorrhoeae* and *E. coli* is greatly influenced by the mode of preparation. Similar variations were seen by Duguid *et al.* (1966) when different culture conditions were applied. These authors discussed their findings in relation to the changing external factors and to ageing processes and the infectious potentials of the bacteria. Our results show that preparation conditions also play an important part in the ultrastructural appearance of the outer bacterial zone.

Conclusion

From these experiments and a consideration of the factors influencing the pilation we were not able to endorse the opinion that simple electronmicroscopical examination of negatively stained gonococci permits definite determination of the strain investigated. The fact that (according to Haustein and Uehrlings) 20% of asymptomatic cases of gonorrhoea are caused by non-piliated organisms does not necessarily indicate infection with type 3 or 4 gonococci. Too many factors influence the morphological appearance of pili, and many more compartments of the glycocalyx, besides the pili, are necessary to

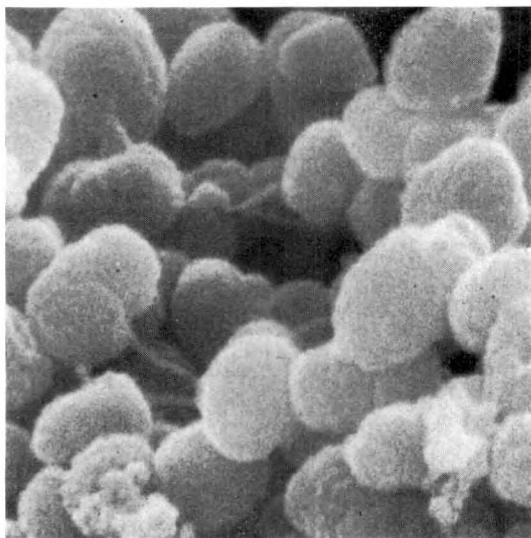


Fig. 3 Scanning electronmicrograph of critical-point dried gonococci on millipore filter; only a few filamentous structures are present between the cells ($\times 19\,000$ magnification)

build up a suitable environment for the multiplication of the bacteria and to form the aggregates called 'infection units' (Novotny *et al.*, 1975).

Electronmicroscopical examination of micro-organisms has made an important contribution to our present knowledge of bacterial structure, and

further progress is to be expected. Further standardisation of the preparation techniques, however, are necessary before typing and quantification of gonococcal pili can be used to determine the infectivity of the strain on a purely morphological basis.

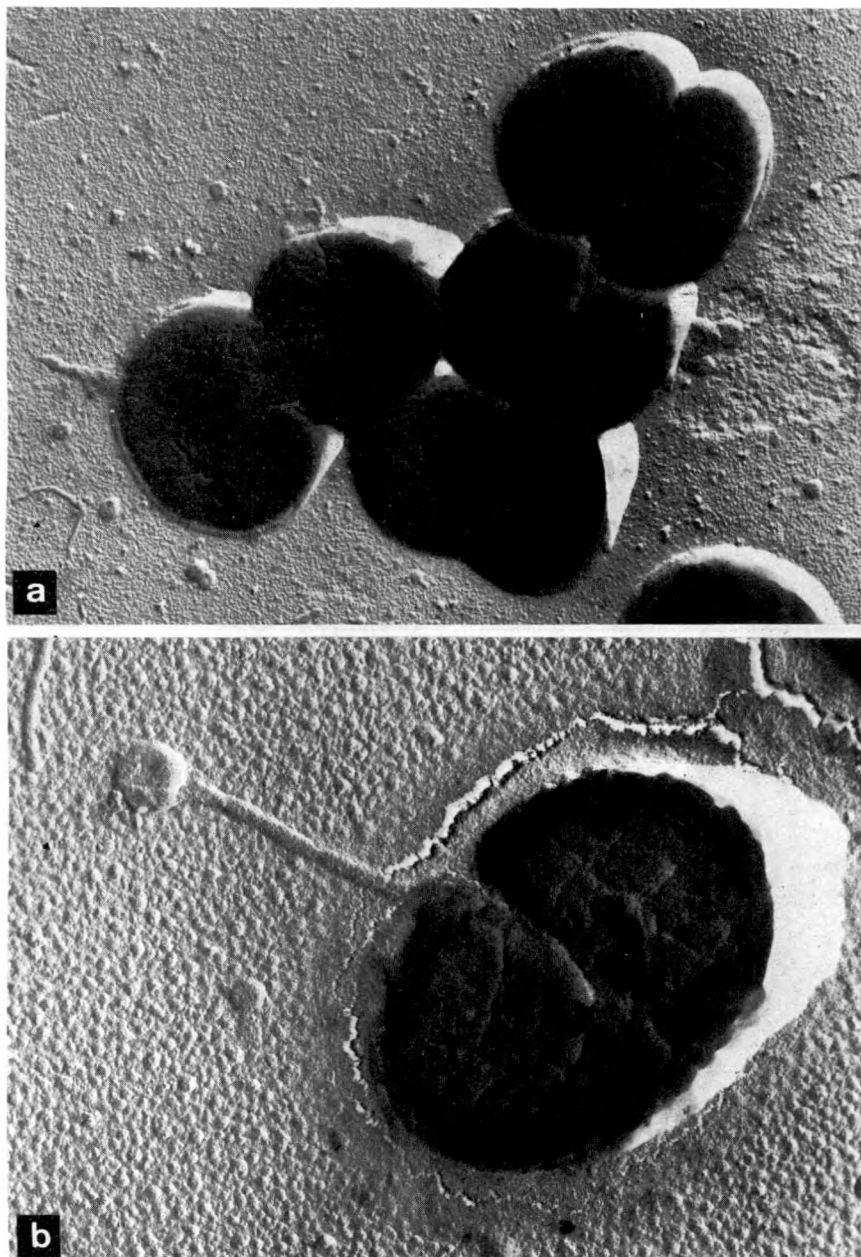


Fig. 4 Carbon-platinum shadowed preparations of snap-frozen gonococci: (a) no pili can be recognised in this specimen ($\times 20\,500$ magnification); (b) a thick filament with a knob at its end seems to extend from the cell ($\times 51\,500$ magnification)

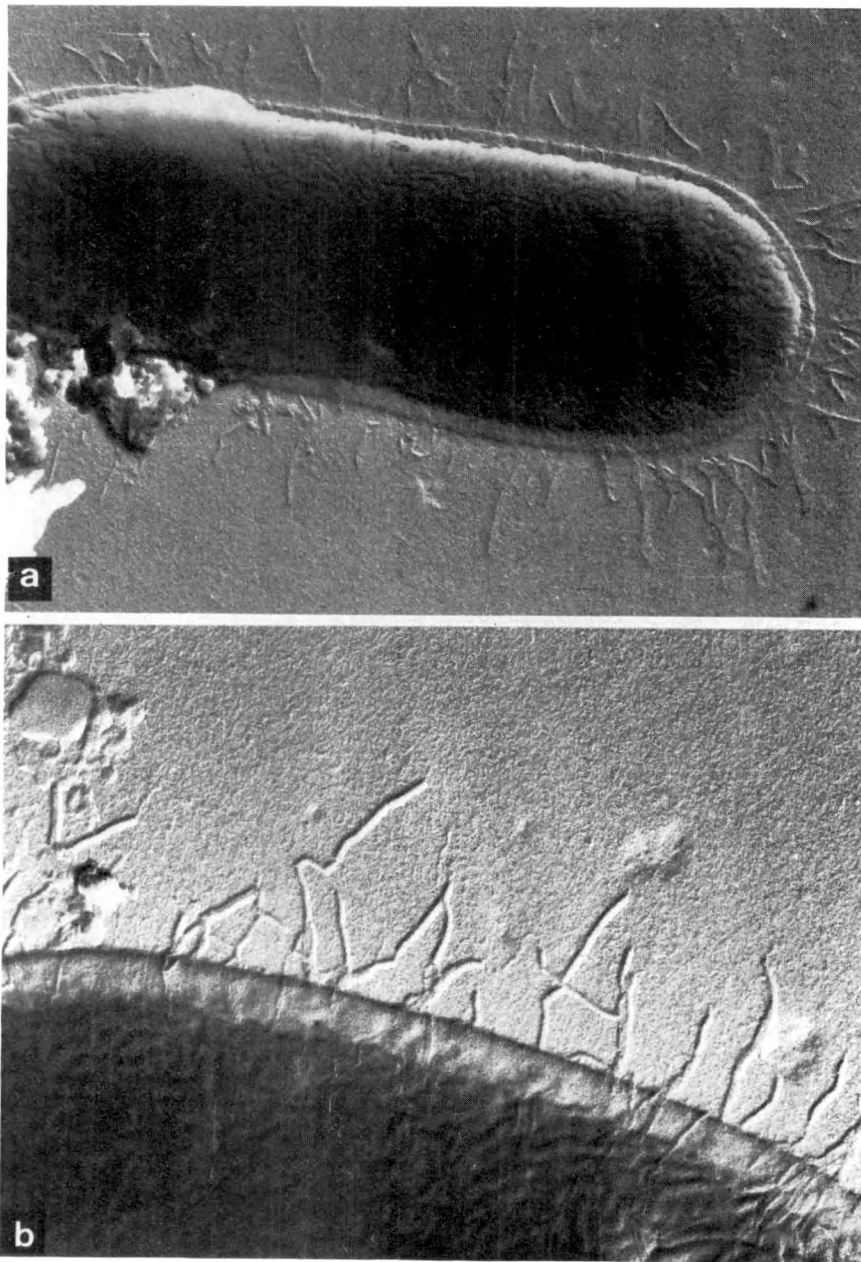


Fig. 5 Carbon-platinum shadowed preparations of snap-frozen coliform bacilli: (a) numerous, short and thin pili are visible ($\times 27\,000$ magnification); (b) numerous pili covering the surface are shown ($\times 61\,500$ magnification)

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Preservation of *Neisseria gonorrhoeae* by the gelatin-disc method

SHIRO YAMAI, YASUSHI OBARA, TAKAYASU NIKKAWA, YUKO SHIMODA, AND YASUSHI MIYAMOTO

From the Department of Bacteriology and Pathology, Kanagawa Prefectural Public Health Laboratory, Yokohama, Japan

SUMMARY Studies of *Neisseria gonorrhoeae* are difficult to perform because of the organism's poor survival *in vitro*. To solve this problem we tried to preserve the organism by a gelatin-disc method. The rate of survival and changes of variations in some biochemical properties of eight strains of *N. gonorrhoeae* were followed for three years. These studies proved that preservation was satisfactory with only a 1/10 reduction of the living cells. Another trial showed that the organism survived for over six months after being frozen at -20°C . The colonial types, agglutination against red cells from rabbit and guinea pig, and antibiotic susceptibility to penicillin, chloramphenicol, tetracycline, kanamycin, and streptomycin did not change after three years' preservation.

Introduction

Kellogg *et al.* (1963, 1968) demonstrated a close correlation between colonial type and pathogenicity of *Neisseria gonorrhoeae*. Later Swanson *et al.* (1971) detected pili in pathogenic T1 and T2 colonies. Correlation between pathogenicity and colonial types and pili has also been reported by several investigators (Thongthai and Sawyer, 1973; Punsalang and Sawyer, 1973; Bumgarner and Finkelstein, 1973). Difficulties have been experienced in such studies, however, because the organism can only survive for a short time and mutation readily occurs with successive transfer of the subculture with loss of pathogenicity.

The most effective method for preserving *N. gonorrhoeae* now in common use is lyophilisation. Its application for a large number of strains, however, is complex and impossible in a small laboratory. We, therefore, attempted and successfully improved the preservation method by using desiccated gelatin, which was originally developed and effectively applied to the species of *Enterobacteriaceae* by Stamp (1947).

Material and methods

MEDIA

Transgrow medium (Martin and Lester, 1971) for primary isolation and Difco GC medium base with 2% defined supplement (GCB-2DS) (Kellogg *et al.*, 1963) for confirmation of colonial-type and cell count were used.

GONOCOCCAL STRAIN AND COLONIAL TYPE

Eight fresh isolates of *N. gonorrhoeae* isolated in our laboratory in 1973-74 were used; strains 48-14 and 48-17 were isolated in 1973 from women with acute cervicitis. Strains 49-2, 49-13, 49-48, 49-52, and 49-60 were all isolated from men with acute urethritis and strain 49-8 was isolated from a woman with chronic cervicitis. The last six strains were isolated in 1974. They were identified by Gram staining, oxidase reaction, and carbohydrate fermentation. The colonial type was determined according to the definition of Kellogg *et al.* (1963) with the colony grown on GCB-2DS medium.

REAGENTS

Reagents for preparing gelatin-disc were prepared as follows:

- (1) Distilled water solution of Bacto dextrose (5%) with Bacto skim milk (3%) and decolouring carbon (0.6%, Norit, Extra, NV

Address for reprints: Dr Y. Miyamoto, Department of Bacteriology and Pathology, Kanagawa Prefectural Public Health Laboratory, 52 Nakaocho Asahi-Ku, Yokohama, Japan

Received for publication 5 September 1978

Norit-Vereeniging) was sterilised at 110°C for 10 minutes and stored in a refrigerator (solution A).

- (2) Distilled water solution of sodium L-ascorbate (0.5%, Wako) was sterilised by filtration through a millipore filter and stored at -20°C (solution B).
- (3) Distilled water solution of Bacto gelatin (20%) was sterilised at 121°C for 15 minutes and stored in a refrigerator (solution C).

As an antioxidant L-ascorbic acid, instead of sodium L-ascorbate, was also used for comparison, but the results were not promising.

INSTRUMENTS AND MATERIALS FOR PREPARING DISCS

Circular filter papers 7 cm in diameter (Toyo no. 2) were soaked in melted solid paraffin (melting point 56–58°C) (Wako) heated at 140°C for five minutes over a gas burner, removed with sterile forceps, and placed individually in a sterilised Petri dish, which was used as the receiver for droplets of cell suspension.

A glass desiccator with 500 g silica gel was used for drying; a glass cylinder with a wide opening containing 200 to 300 g of phosphorus pentoxide (P_2O_5) was placed in the desiccator.

A sterile capillary pipette giving a 0.03-ml droplet was used for dropping the cell suspension.

A 5-ml small vial with a wide opening, containing silica gel, was closed with a cotton plug and used for the storage of the desiccated gelatin discs containing the organisms.

PREPARATION OF GELATIN DISC

Each of the eight strains was inoculated on to two to three GCB-2DS agar plates and incubated at 35°C for 18 to 20 hours in a candle extinction jar. The organisms were collected to prepare a homogeneous suspension of 10^{10} cells/ml of solution A previously warmed to 35°C and stirred for 30 seconds with a vibrator. To this suspension were added similarly warmed 0.2 ml of solution B and 1 ml of solution C, which had been previously dissolved by heating and kept at 35°C; the suspension was stirred again for 30 seconds. The mixed suspension thus prepared was delivered dropwise with the capillary pipette on to the paraffin-soaked filter paper in the Petri dish. This Petri dish, with its cover slightly open, was placed in the desiccator and dried at room temperature under reduced atmospheric pressure (20 mm Hg [2.7 kPa]) with a vacuum pump. After four to six hours the hydrated portion near the surface of the P_2O_5 was removed to help further dehydration. It was then left overnight under a reduced pressure of 10 mm

Hg (1.35 kPa). After 24 hours the droplet had completely dried and changed into a thin and flat circular gelatin disc, which either came off the filter paper spontaneously or was lifted off with forceps. The discs of each strain were collected and put in the small vial, which was then tightly sealed and stored at -20°C.

VIABLE CELL COUNT

The quantitative cell count was performed on strains 48-14 and 48-17 immediately before and after drying and every month of each year thereafter. In addition, a pool of five discs from each strain was put into a small test tube, dissolved, and resuspended with 1 ml of heart infusion broth (Eiken) by heating at 35°C. After a series of tenfold dilutions of this original suspension had been prepared, each 0.1 ml of the dilution was streaked on a GC-2DS agar plate and then incubated at 35°C in a candle extinction jar for 24 hours. The number of colony-forming units (cfu) was thus counted, giving the number of viable cells per disc. In addition, six strains (49-2, 49-8, 49-13, 49-48, 49-52, and 49-60), were subjected to a qualitative study—that is, one disc from each strain was dissolved in 0.2 ml of heart infusion broth, one loopful of which was streaked on to a GCB-2DS agar and incubated at 35°C for 24 to 48 hours to determine colony-forming capacity.

STORAGE TEMPERATURE

To examine the effect of temperature on the survival of cells embedded in the gelatin disc, discs from the same batch were stored at different temperatures (that is, at 30°C, 5°C, and -20°C) and the change in cfu was followed with estimations at weekly intervals for six weeks and finally at 24 weeks.

ANTIBIOTIC SUSCEPTIBILITY TESTING

The method of Martin *et al.* (1970) was used for testing antibiotic susceptibility to penicillin, chloramphenicol, tetracycline, kanamycin, and streptomycin.

HAEMAGGLUTINATION REACTION

Red cells from rabbit and guinea pig were used according to the method of Punsalang and Sawyer (1973).

Results

All the eight strains stored survived three years' preservation. The decline of viable cells of strains 48-14 and 48-17 are shown in Figure 1. Despite a

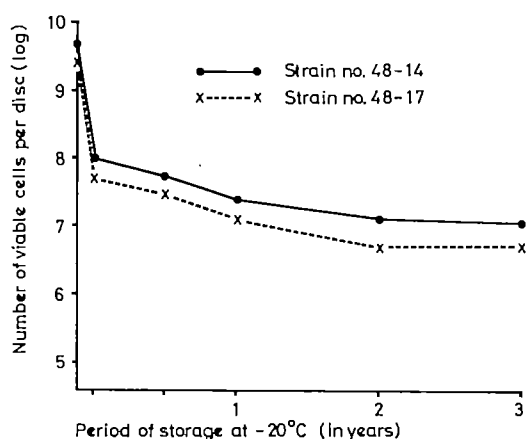


Fig. 1 Survival of *Neisseria gonorrhoeae* in gelatin discs

rapid decline of viable cells up to 10^{-3} of the initial suspension immediately after desiccation, the decline was less thereafter and dropped within the range of 10^{-1} of the initial suspension. The decline of viable cells, therefore, throughout the three-year preservation period was within the order of 10^{-3} of the initial suspension.

As shown in Table 1, colonial types were also maintained well after three years' preservation with only a slight decrease in frequency of appearance of the types 1 and 2 of some strains.

Tests of the effect of temperature on the viability of the cells embedded in gelatin disc showed that they were least affected when stored at -20°C even after six months, whereas no colony was formed after five to six weeks when stored at 30°C . The decline of viable cells after six months was within the order of 10^{-3} at -20°C and 10^{-4} at 5°C .

As regards the effect of antioxidants added to the mixed suspension, the decrease in viable cell count

immediately after freezing was less with sodium L-ascorbate than with L-ascorbic acid—that is, the former decrease was 10^{-3} whereas the latter was 10^{-5} of the initial suspension. The decrease while freezing was also less in the case of sodium L-ascorbate, the minimum effect being with the addition of 0.05 ml/ml giving a final concentration of 0.5%, and it was, therefore, appropriate for use. These effects are shown in Figure 2.

As shown in Table 2, no change was observed in the antibiotic susceptibility of any strains which were either highly sensitive or relatively resistant to penicillin or sensitive or resistant to streptomycin. They all agreed within a one-step difference of the gradation of minimum inhibitory concentration (MIC) after three years' preservation. Similarly, no change was observed in the susceptibility to chloramphenicol, tetracycline, and kanamycin.

No changes were seen in haemagglutination reaction when blood cells of either rabbit or guinea pig were used.

Comment and conclusions

Lyophilisation has been regarded as the most effective method for preserving strains of *N. gonorrhoeae*. Convenient methods have also been reported by Pospisil and Kabátová (1969), Sparling (1966), Thomas *et al.* (1973), Ofek *et al.* (1974),

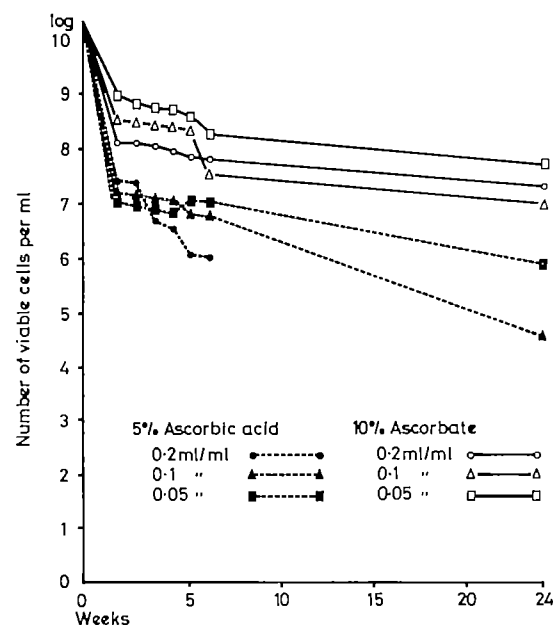


Fig. 2 Effect of antioxidants (sodium L-ascorbate and L-ascorbic acid) on survival of *Neisseria gonorrhoeae* during 24 weeks of preservation by freezing at -20°C

Table 1 Percentage of colonial types of original and recovered cultures

Strain no.	% of colonial types			
	Original culture		Recovered culture (after 3 years)	
	Type 1-2	Type 3-4	Type 1-2	Type 3-4
48-14	10	90	10	90
48-17	10	90	10	90
49-2	90	10	90	10
49-8	90	10	80	20
49-13	90	10	90	10
49-48	90	10	70	30
49-52	90	10	80	20
49-60	90	10	90	10

Table 2 Drug susceptibility of strains of *Neisseria gonorrhoeae* before and after storage for three years

Strain no.	Year	Drug susceptibility (MIC)				
		PC (μ g/ml)	SM (μ g/ml)	CP (μ g/ml)	TC (μ g/ml)	KM (μ g/ml)
48-14	1974	0.03	6.3	4	1	8
	1977	0.02	12.5	4	1	4
48-17	1974	1	>200	2	1	8
	1977	0.05	>200	2	1	4
49-2	1974	2	>200	8	4	16
	1977	1	>200	8	4	16
49-8	1974	0.02	6.3	4	1	8
	1977	0.03	6.3	4	1	8
49-13	1974	1	>200	4	2	8
	1977	1	>200	4	4	16
49-48	1974	1	>200	8	4	8
	1977	1	>200	8	4	8
49-52	1974	1	>200	2	0.5	8
	1977	1	>200	2	1	8
49-60	1974	1	>200	8	2	8
	1977	2	>200	8	2	16

PC penicillin, SM streptomycin, CP chloramphenicol, TC tetracycline, KM kanamycin

and Apicella (1974). These lyophilisation or freezing methods, however, require expensive instruments and considerable space is occupied by the ampoules or tubes, which limits the number of strains to be stored.

In order to preserve strains of *N. gonorrhoeae* we attempted to apply and modify Stamp's (1947) classic simple method of preserving bacteria embedded in dried gelatin. For this purpose we used a lyophilisation medium, which we have routinely used for *Vibrio cholerae* strains, to which gelatin was added. The experiment resulted in success and *N. gonorrhoeae* was stored and kept alive by our gelatin-disc method for over three years. The properties which the organisms possessed at the time of fresh isolation were retained. Gelatin-mixed bacterial suspensions used for preparing the gelatin discs could also be stored in a viable state without desiccation by being frozen at -20°C for over six months.

Since our method is simple and requires neither expensive instruments nor large storage space, it is applicable in a small laboratory. The preservation of numerous strains under the same conditions in a compact state with many but small discs enables us to repeat our experiment and makes a long-term experiment feasible under the same conditions. Since the organism can also survive in gelatin discs at room temperature for several weeks, the method is simple and convenient for transportation.

When gelatin discs are prepared, the following should be observed:

- (1) Large amounts of a young culture incubated for 18 to 20 hours should be used.
- (2) A dense cell suspension, such as 10^{10} cells per ml, should be prepared.
- (3) Desiccation should be completed within a short time. The hydrated portion of P_2O_5 should, therefore, be removed and replaced with fresh P_2O_5 during the drying process.
- (4) Once desiccation is complete, the discs should be stored in a small vial with silica gel—that is, under dehydrated conditions at 5°C or -20°C .

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Theme 2: Recent advances in the immunology of syphilis

Role of humoral versus cellular mechanisms of resistance in the pathogenesis of syphilis

M. METZGER

From the Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland

SUMMARY From experimental work to determine the role of humoral and cell-mediated responses in the pathogenesis of syphilis evidence has been collected to indicate that both humoral and cell-mediated immunity have a protective function in syphilis.

Introduction

It is well known that both rabbits and man infected with *Treponema pallidum* develop resistance to reinfection during the course of the disease (Turner and Hollander, 1957). It has also been shown that a marked resistance to challenge inoculation can be achieved in rabbits by immunisation with the virulent Nichols strain of *T. pallidum* rendered non-infectious either by storage (Metzger *et al.*, 1969; Metzger and Smogór, 1969) or by gamma irradiation (Miller, 1973). The mechanisms underlying immunity in syphilis, however, are poorly understood. Multiple antibodies circulate in the disease, and recent evidence shows that a cell-mediated response develops during the infection. To what extent these two immunological responses protect against syphilis is not clear. The aim of this paper is to give a concise review of the research efforts aimed at clarifying this point.

Humoral immunity

Studies aimed at determining the role of antibodies, or more strictly speaking the role of humoral factors, began in the 1930s and have continued until recently. Experiments of this kind can be divided into three groups: (1) those designed to detect treponemicidal activity in syphilitic serum; (2) those aimed at finding a correlation between the occurrence and level of antibodies and the state of resistance; and (3) those attempting a passive transfer of immunity by immune serum.

Address for reprints: M. Metzger, Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, 53-114 Wrocław, Poland

Received for publication 5 July 1978

TREPONEMICIDAL ACTIVITY IN SYPHILITIC SERUM

In his pioneering work Turner (1939) showed that treponemes which had been incubated with sera from untreated syphilitic rabbits lost their virulence and were unable to produce syphilitic lesions. Normal rabbits which had been challenged intracutaneously with such treated treponemes usually failed to develop lesions, or else the resulting lesions had a longer incubation period and remained smaller than those produced by treponemes incubated with normal serum. Later, Turner *et al.* (1948) used serum from syphilitic and non-syphilitic human beings, which gave similar results. Sera from patients with secondary, latent, or tertiary syphilis exerted an inhibitory effect on the development of syphilitic lesions, whereas sera from non-syphilitic persons did not.

These investigations led to the development of the *T. pallidum* immobilisation (TPI) test. In recent years, the treponemicidal activity of syphilitic serum has been studied extensively by Bishop and Miller (1976b) in Los Angeles. Factors have been demonstrated in immune serum which inactivate *T. pallidum* and render it incapable of producing symptomatic infection in rabbits; the appearance, level, and persistence of the factors were found to correlate closely with the development of resistance to symptomatic reinfection after initial *T. pallidum* infection of the source rabbits.

HUMORAL ANTIBODIES AND RESISTANCE AGAINST SYPHILIS

In other studies a correlation was sought between the appearance and level of antibodies reacting in various serological tests and resistance to syphilitic

infection. Experiments of this kind were performed in several laboratories and all gave negative results (Izzat *et al.*, 1971; Magnuson *et al.*, 1951; McLeod and Magnuson, 1953; Metzger *et al.*, 1969; Metzger and Smogór, 1969; Miller *et al.*, 1963). None of the investigators could show a correlation between the degree of resistance and the titre of antibodies, even in the case of antibodies clearly associated with resistance, such as immobilisins. Our own findings also show this lack of correlation (Table 1).

A total of 302 rabbits were immunised with a treponemal vaccine developed in our laboratory (Metzger and Smogór, 1969). On the basis of the results of the infectivity test the rabbits were classified as completely immune, partially immune, and non-immune. Assay of circulating antibodies was conducted repeatedly during immunisation and after challenge by a variety of serological tests for syphilis. Only those results, however, which were obtained five weeks after completion of the immunisation (that is, just before challenge inoculation) are recorded in Table 1. Both the mean values and ranges of serum titres found in the various serological tests were nearly the same in the three groups of rabbits, which showed different degrees of susceptibility to infection. It was particularly interesting to note that none of the immunised rabbits responded with the production of TPI antibody—not even those which proved completely resistant to challenge. Close inspection of the pertinent protocols has also shown no relation between the serological response of individual animals and their immune status; rabbits whose sera exhibited the same or nearly the same degree of serological reactivity behaved differently on challenge, being either completely free of syphilitic infection or showing asymptomatic or even symptomatic infection.

PASSIVE TRANSFER OF RESISTANCE BY IMMUNE SERA

In recent years the most extensive studies of this type were carried out in rabbits by Bishop and Miller (1976a), Perine *et al.* (1973), Sepetjian *et al.*

(1973), and Weiser *et al.* (1976). Their results were similar; injections of hyperimmune serum delayed the appearance and diminished the severity and duration of syphilitic lesions which developed after challenge with *T. pallidum*. Thus, these studies have suggested that a humoral mechanism of resistance may have been transferred by passive immunisation, despite vast amounts of serum (ranging up to 400 ml per rabbit) and prolonged daily administration (over 37 days) having been needed to bring about this incomplete protective effect.

SUMMARY OF ROLE OF HUMORAL FACTORS

Syphilitic serum exerts a lethal effect on treponemes and immunity to syphilis can be passively transferred by immune serum; no true correlation, however, has been established between the appearance and level of a variety of syphilitic antibodies—even those clearly associated with resistance—and the degree of resistance. Despite this limitation these findings have suggested that humoral immunity is operative in syphilis. No evidence has been shown, however, that antibodies are concerned in the immune reaction and no protective antibody has been demonstrated.

Cell mediated immunity

Preliminary experiments have been carried out to discover whether and how the cell-mediated response to treponemal antigens develops in rabbits during syphilitic infection and following artificial immunisation (Metzger *et al.*, 1977). The macrophage migration inhibition (MMI) test was used to demonstrate this type of immunological response.

Lymphocytes of syphilitic rabbits exhibited a well marked capacity to inhibit macrophage migration as early as one month after onset of the infection (Table 2). The level of this response rose slowly during the next two months, reaching an average value of about 50% inhibition in the third month. Between the fourth and sixth months,

Table 1 Results of studies to determine correlation between syphilis immunity and humoral antibody response

Immune status of rabbits*	Reciprocal serum titres in serological tests											Opsonophagocytic test (% positive sera)	
	VDRL		TPI		FTA		Agglutination		Antiglobulin agglutination		TPHA		
	Mean	Range			Mean	Range	Mean	Range	Mean	Range	Mean		Range
Immune	2	0-32	0	54	0-320	186	0-1280	80	0-160	170	0-2560	52	
Partly immune	3	0-64	0	57	0-320	180	0-1280	64	0-80	200	0-2560	31	
Non-immune	2	0-32	0	43	0-320	110	0-640	108	0-320	170	0-1280	42	

*Immune means no lesions and lymph node transfers negative; partly immune, no lesions but lymph node transfers positive; and non-immune, lesions at one or more challenge sites

Table 2 *MMI response in rabbits experimentally infected with Treponema pallidum*

Months after infection	No. of rabbits tested	Mean % inhibition	SD
1/2	16	4.7	10.5
1	18	42.9	19.7
2	18	37.2	21.6
3	14	46.3	18.4
4	19	19.8	20.7
6	11	4.8	9.5
8	16	45.8	19.2
11	10	49.7	15.2
12	10	48.3	13.9
13	11	54.2	16.5
15	10	68.2	11.8
16	9	72.0	15.5
26	6	74.3	9.2

however, a distinct weakening of this capacity occurred, followed by a slow but steady increase during the next two years, that is, until the end of the observation period.

The development of the MMI response in rabbits artificially immunised with non-viable treponemes is shown in Table 3. As early as four weeks after the start of immunisation, the lymphocytes of the rabbits exerted a marked inhibitory effect on the migration of macrophages. This capacity reached a peak value (about 40% inhibition) by the second month, remained at this level with slight fluctuations during the subsequent six months and thereafter showed a slow decrease.

These results clearly show that during syphilitic infection and following artificial immunisation the cell-mediated response to treponemal antigens develops in the host. The demonstration of this type of immunological response does not, however, directly indicate that this reaction plays a protective role in syphilis. Thus, to show this, experiments were designed following essentially the same research plan as that used for demonstrating the role of humoral factors.

Table 3 *MMI response in rabbits immunised with non-viable Treponema pallidum*

Months after start of immunisation	No. of rabbits tested	Mean % inhibition	SD
1	16	29.2	16.4
2	17	39.6	18.2
3	14	35.5	14.6
4	5	37.4	16.2
6	5	35.4	12.7
7	5	34.8	13.4
8	5	33.2	12.5
10	4	19.2	8.4
11	6	16.3	6.8

TOXIC EFFECT OF IMMUNE LYMPHOCYTES

The results of these studies showed that extracts of lymphocytes from immune rabbits exerted an *in-vitro* toxic effect on *T. pallidum* whereas those from non-immune animals did not (unpublished data).

MACROPHAGE MIGRATION AND RESISTANCE TO INFECTION

Attempts to correlate the appearance and level of the cell-mediated immunological response with resistance to infection gave rather equivocal results (Metzger *et al.*, 1977) (Table 4). After being immunised, a group of 11 rabbits was infected intradermally with *T. pallidum*. The MMI test was performed once during the immunisation course and twice after completion. The results of the test, performed just before challenge, were compared with the immune status of the animals, as shown by the infectivity test. The post-immunisation level of the MMI response varied greatly in individual animals, the percentage inhibition ranging from 0% to 73% (Table 4). When the results of the MMI test are compared with those of the infectivity test, however, no strict correlation was shown between the level of the MMI response of the individual animals and their ability to combat the infection. Rabbits whose lymphocytes inhibited migration of macrophages to a varying degree, and even the rabbit (no. 2100) whose lymphocytes did not exhibit this ability on repeated testing, proved immune or partly immune after challenge. As a group, however, the lymphocytes from immune and partly immune rabbits exerted a much greater

Table 4 *Results of attempt to correlate levels of MMI response with resistance to infection with Treponema pallidum*

Rabbit no.	% inhibition (in weeks after start of immunisation)			Immune status*
	4	8	11	
2118	16	75	73	immune
2121	12	44	56	
2152	12	53	47	
2143	0	38	45	
2122	0	45	38	
2100	6	0	0	partly immune
724	32	54	46	
2101	45	50	42	
2040	53	54	29	
2054	37	31	10	non-immune
2133	0	0	0	

*Immune means no lesions and lymph node transfers negative; partly immune, no lesions but lymph node transfer positive; non-immune, lesions at one or more challenge sites

- inhibitory effect on the migration of macrophages compared with those from rabbits of the non-immune group.

EFFECT OF IMMUNOSUPPRESSION

The clinical course of syphilis has been compared in rabbits treated with immunosuppressive agents (Pacha *et al.*, 1978) and in those not so treated. These experiments were designed on the assumption that a milder course of the disease in the rabbits with experimentally induced immunosuppression would indicate a pathogenic role of humoral or cell-mediated immunity or both. On the other hand, a more severe course of the disease under these experimental conditions could be interpreted as evidence of the protective role of humoral or cell-mediated immunity or both. The results of this study suggested the latter possibility.

Treatment of syphilitic rabbits with immunosuppressive agents (cyclophosphamide or 5-fluorouracil) resulted in almost complete abolition of the cell-mediated response (as measured by the MMI test), while the humoral response (as measured by the Venereal Disease Research Laboratory (VDRL), fluorescent treponemal antibody (FTA), and *T. pallidum* haemagglutination (TPHA) tests), was not affected by this procedure (Table 5). In the group of rabbits given immunosuppressors syphilitic lesions appeared not only at the challenge sites as usual but, after healing of the primary lesions, multiple multiform secondary lesions developed over the entire dorsal area. These results may be interpreted as demonstrating the protective role of the cell-mediated response in syphilis.

TRANSFER OF RESISTANCE BY IMMUNE LYMPHOCYTES

The possibility of such a protective role has been reinforced by experiments in which immunity was successfully transferred from immune donor rabbits to normal recipient rabbits with lymphocytes extracted from the lymph nodes of immune donors (Metzger and Smogór, 1975). Lymphocytes from those donors which had had previous experience with *T. pallidum*, (that is, those immunised with non-viable *T. pallidum* or cured of syphilitic infection by penicillin treatment) conferred a state of resistance to challenge on most of the recipient rabbits (Table 6); this was demonstrated either by the absence of lesions at the challenge sites or by the reduced number and delayed incubation period of lesions compared with those in the controls. Some of the donor and recipient rabbits were also tested for humoral and cell-mediated response by the TPI and MMI tests respectively. The lymphocytes of the donors were active in inhibiting migration of macrophages, and this ability was found in the recipients after lymphocyte transfer (Table 6). In contrast, however, although the rabbits of the immunised group responded with TPI antibody this response could not be transferred by lymphocytes into recipient rabbits.

Although these experiments could not be performed under conditions which guaranteed the survival of the transferred lymphocytes in recipient rabbits (syngeneic rabbits were not available), evidence of partial protection was achieved in most of the passively immunised animals. Furthermore, every step of the procedure used in this study was

Table 5 Clinical course of syphilis in immunosuppressed rabbits

Syphilitic rabbits	Humoral response (VDRL, TPI, FTA, TPHA tests)	Cell-mediated response (MMI test)	Clinical course
Untreated	+	+	Typical syphilitic lesions at challenge sites
Treated with immunosuppressors (cyclophosphamide or 5-fluorouracil)	+	0	Exacerbated (multiple disseminated secondary syphilitic lesions)
+ Positive			

Table 6 Transfer of syphilitic immunity by immune lymph node lymphocytes

Donor rabbits				Recipient rabbits			
Pretreatment	No. of rabbits	Humoral response (TPI test)	Cell-mediated response (MMI test)	No. of rabbits	Humoral response (TPI test)	Cell-mediated response (MMI test)	No. of immune rabbits
Immunised with non-viable <i>T. pallidum</i> or infected with <i>T. pallidum</i> and cured	17	+	+	19	0	+	16
None	9	0	0	9	0	0	0

+ Positive

arbitrarily established; different experimental conditions may give more complete protection and this is now being studied in our laboratory. Despite these limitations, the findings of this study together with those previously described strongly indicate the protective role of cell-mediated immunity in syphilis. Baughn *et al.* (1977), however, reported the inability of spleen cells from immune rabbits to confer immunity to challenge with *T. pallidum*. I think that this failure will be clarified by further experiments on a more extended basis.

Conclusion

Both humoral and cell-mediated responses are concerned in the pathogenic mechanisms in syphilis. Humoral and cell-mediated immunity, however, can be separated only for purposes of analysis, since in the organism they act in concert to protect the host against infection. The interplay between humoral and cellular immunity is important in producing the great variability of clinical symptoms in syphilis and may even contribute to the recurrence of the disease.

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Attenuated yaws in Surinam

P. L. A. NIEMEL,* E. A. BRUNINGS,† AND H. E. MENKE‡

From the *Dermatological Service, and the †Professor P. C. Flu Institute, Paramaribo, Surinam; and the ‡Department of Dermatology, University Hospital, Rotterdam (Dijkzigt), The Netherlands

SUMMARY Results of a pilot survey in the central, northern region of Surinam indicate that yaws is prevalent (hypoendemic) in this part of the country. No classical symptoms of yaws were seen. The observed lesions were scanty or solitary, dry papillomatous, or macular and scaly, which fits into the clinical picture of attenuated endemic treponematoses.

Introduction

Surinam is a tropical country in the north-eastern part of South America. Syphilis and yaws are known to be prevalent in this country (Menke, 1978). Yaws was highly prevalent at the beginning of this century (Flu, 1911). Although no special mass treatment campaign against yaws has ever been organised, a gradual decline in the incidence of the condition was observed over past decades, and now it is generally assumed that the disease has practically been eradicated in Surinam. In 1975, attention was focused again on this non-venereal treponemal disease when several children from the central, northern region of the country (Saramacca district) were seen with early infectious yaws. In view of this we carried out a pilot survey to study the prevalence of this disease in that part of the country.

Material and methods

In 1976, a total of 969 schoolchildren living in the central, northern region (Saramacca district) of Surinam (age range 4-16 years, mean 11 years) were examined with special attention being given to skin and mucous membranes. Blood samples were collected by venepuncture. The Venereal Disease Research Laboratory (VDRL) slide test was performed at the Professor P. C. Flu Institute in Paramaribo according to the recommended procedure (National Communicable Disease Center, 1969). The results were scored as positive (reactive) or negative (non-reactive). The remainder of the serum was stored at -70°C for future analysis (Menke *et al.*, 1979). The housemates of the

schoolchildren with reactive VDRL test results were examined and the VDRL test was also performed in these cases (epidemiological investigations). Skin specimens for biopsy were taken from individuals with lesions suggesting yaws, and sections were stained with haematoxylin-eosin (routine) and silver (for treponemes). All VDRL-reactive children (as well as their VDRL-non-reactive housemates) were treated with a single injection of benzathine penicillin G, in doses which were adjusted according to age: children of 4-6 years, 0.6 megaunits; children of 7-12 years, 1.2 megaunits; and children older than 12 years, 2.4 megaunits.

The diagnosis of yaws was based on a combination of epidemiological data, clinical findings, results of the VDRL test, histological evidence, and the effect of treatment with penicillin.

Results

Of the 969 children examined, 212 (22%) had a reactive VDRL test, and 29 of these showed clinical lesions suggesting early yaws. The lesions observed were either solitary, dry papilloma-like (Fig. 1), or macular and scaly (Fig. 2). The lesions disappeared after treatment with penicillin. Biopsy of papilloma-like lesions showed moderate acanthosis of the epidermis, a moderate inflammatory infiltrate consisting of neutrophils in the epidermis, and lymphocytes and many plasma cells in the dermis. Proliferation of small vessels and some prominence of endothelial cells were observed. Spiral structures suggesting treponemes could be identified by silver-staining, especially in the epidermis (Fig. 3). These findings are consistent with the diagnosis of yaws. In patients with macular, scaly lesions, the histological picture showed a non-specific dermatitis consisting of parakeratosis, acanthosis of the epidermis, and a perivascular infiltrate of lympho-

Address for reprints: Dr H. E. Menke, National Institute of Public Health, PO Box 1, Bilthoven, The Netherlands

Received for publication 3 October 1978



Fig. 1 Papilloma-like lesions in the perianal region of a 9-year-old boy with secondary yaws



Fig. 2 Scaly macules on the palms of a 10-year-old boy with secondary yaws

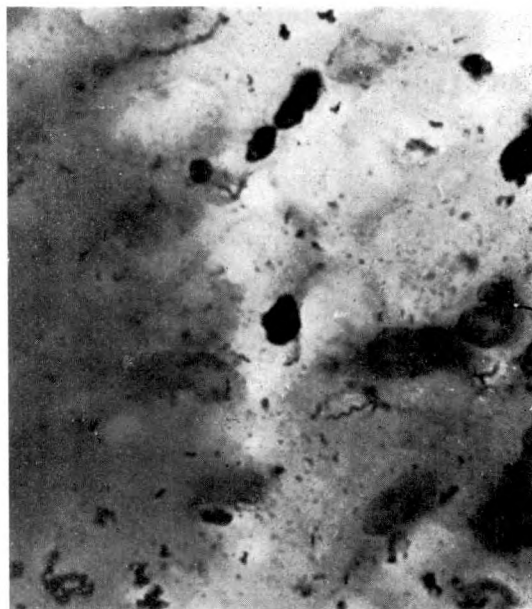


Fig. 3 Spiral structures suggesting treponemes in the epidermis of the lesions shown in Fig. 1 (silverstain $\times 1100$ magnification)

cytes in the dermis. Treponemes could not be identified in these cases.

Signs of congenital syphilis and genital lesions suggesting venereal syphilis were not seen in any of the children. Of the 29 individuals with lesions, 15 were classified as having primary yaws and 14 as having secondary yaws. In the 183 children with positive results to the VDRL test but without clinical lesions a presumptive diagnosis of latent yaws was made.

Discussion

The results of this study indicate that yaws is still prevalent in the central, northern region of Surinam. According to World Health Organisation standards (Hackett and Guthe, 1956) the prevalence of the disease in this region can be classified as hyp endemic.

In textbooks classical lesions of yaws are described as multiple, papillomatous, moist lesions present all over the body (Rook *et al.*, 1972; Canizares, 1975). Flu (1911) also observed these classical lesions 70 years ago in individuals in the central, northern region of Surinam. Recently, similar lesions have been seen in patients coming from other foci of yaws in the country (Niemel *et al.*, unpublished data). In this survey, however, carried

out in the central, northern region of Surinam, we did not see classical yaws lesions. The lesions observed by us were scanty or solitary, squamous, or dry papillomatous. These findings fit into the clinical picture of attenuated yaws (Vorst, 1974). The cause of this attenuation is not known, but attenuated treponemal disease may be related to low endemicity, and classical yaws is seen in hyper-endemic areas (Vorst, 1974).

The prevalence of attenuated yaws may have practical consequences and the diagnosis can be easily overlooked. Physicians, therefore, should be aware of the existence of these atypical cases. Furthermore, serological investigation is essential to make the right diagnosis.

The fact that yaws is still prevalent in Surinam is not only of importance to local physicians and health authorities. In addition to the 400 000 inhabitants of Surinam over 100 000 people of Surinamese origin live in Holland. Most of them emigrated to that country shortly before the independence of Surinam in 1975. It is obvious that, together with the people who recently emigrated from Surinam to Holland, yaws infection could have been imported to Holland and hence to Europe. In most cases the infection would probably be latent. European physicians should consider the possibility of active yaws in children with skin disease who have recently come from Surinam and from other tropical countries.

Finally, in view of our findings, health authorities, local and international (such as, the World Health Organisation and the Pan American Health Organi-

sation), are asked to consider setting up a control programme to eradicate yaws in this part of the world.

The authors thank Patricia Oehlers, laboratory technician, Dr A. F. Brathwaite, pathologist (both from the Professor P. C. Flu Institute in Paramaribo), Dr V. D. Vuzevski, pathologist (Pathology Department, Medical Faculty, Erasmus University, Rotterdam) and Dr E. Stolz, dermatologist (Dermatology Department, Medical Faculty, Erasmus University, Rotterdam) for their help and co-operation.

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Comparison of cardiolipin and treponemal tests in the serodiagnosis of yaws

H. E. MENKE,* J. VELDKAMP,* E. A. BRUNINGS,† P. L. A. NIEMEL,‡
A. NOTOWICZ§ AND E. STOLZ§

From the *National Institute of Public Health, Bilthoven, The Netherlands; the †Professor P. C. Flu Institute and the ‡Dermatological Service, Paramaribo, Surinam; and the §Department of Dermatology, University Hospital, Rotterdam (Dijkzigt), The Netherlands

SUMMARY Results of the Venereal Disease Research Laboratory (VDRL), rapid plasma reagin (RPR), *Treponema pallidum* haemagglutination (TPHA), *T. pallidum* immobilisation (TPI), and fluorescent treponemal antibody absorption (FTA-ABS) tests on sera of 661 children from a region where yaws is hypoendemic are compared. For 107 (16.2%) out of 661 sera the FTA-ABS test was the only one showing reactivity; in these instances the test was weakly reactive (intensity of fluorescence scored as +) and the children had no history and no signs or symptoms of treponemal disease. A solitary, weakly reactive FTA-ABS test result seems to have no clinical significance in these cases. The FTA-ABS test can be used as a confirmatory test for yaws instead of the TPI test, if only the results of sera showing an intensity of fluorescence scored as ++ or more are considered to be positive. There appeared to be no significant differences in the results of the VDRL, RPR, and TPHA tests as screening tests for yaws when the TPI or FTA-ABS tests were used as reference tests.

Introduction

The value of various serological tests for the screening and confirmation of syphilis has been fairly well established. The literature on their use for screening and diagnosis of other treponematoses, however, is scanty. This study was carried out to assess the value of cardiolipin and treponemal tests in the serodiagnosis of yaws.

Material and methods

In this study, carried out in 1976 and 1977, initially a total of 969 children living in an area of Surinam where yaws is hypoendemic were examined; clinical and epidemiological data on these children have been given (Niemel *et al.*, 1979). Blood samples from all the children were collected by venepuncture. A part of each serum sample was used in Surinam for the Venereal Disease Research Laboratory (VDRL) test and the remainder was stored at -70°C before being sent to the Netherlands for

other tests. The VDRL slide test was performed at the laboratory of the Professor P. C. Flu Institute, Paramaribo, Surinam, according to the recommended procedure (National Communicable Disease Center, 1969). Reagents were purchased from Baltimore Biological Laboratories (Cockeysville, Maryland 21030, USA).

The rapid plasma reagin (RPR) card test and the *Treponema pallidum* haemagglutination (TPHA) test were performed at the laboratory of the dermatology department, University Hospital, Rotterdam-Dijkzigt, according to manufacturer's manual. RPR kits were purchased from Hynson, Westcott and Dunning Inc. (Baltimore, Maryland 21201, USA). Reagents for the TPHA test (manufactured by Wellcome Reagents Ltd, Beckenham, Kent) were obtained from Wellcome Nederland BV, Amsterdam.

The *T. pallidum* immobilisation (TPI) test and the fluorescent treponemal antibody absorption (FTA-ABS) test were performed at the venereal disease department of the bacteriology laboratory, National Institute of Public Health, Bilthoven, The Netherlands. Both tests were performed according to the recommended procedures (National Communicable Disease Center, 1969) with slight modi-

Address for reprints: Dr H. E. Menke, National Institute of Public Health, PO Box 1, Bilthoven, The Netherlands

Received for publication 3 October 1978

fications; in the TPI test lysozyme (100 µg/ml) was added to the sustaining medium; in the FTA-ABS test the sera were used in dilutions of 1/12 instead of 1/5 after absorption with an ultrasonicate of Reiter treponemes instead of sorbent.

The results of the VDRL, RPR, TPHA, and TPI tests were scored as either positive or negative. The result of the FTA-ABS test was scored as positive when the intensity of fluorescence was ++ or more, weakly positive when the intensity of fluorescence was +, or negative.

For comparing the results of the screening tests (a) (VDRL, RPR, and TPHA tests), the TPI test and FTA-ABS test were used as reference tests (b). The criteria used in this comparison are defined as follows:

(1) Agreement =
$$\frac{\text{number of sera giving identical* results in (a) and (b)}}{\text{total number of sera investigated}} \times 100$$

(2) Sensitivity =
$$\frac{\text{number of sera positive in (a) and (b)}}{\text{total number of sera positive in (b)}} \times 100$$

(3) Rate of possible false-positive reactions =
$$\frac{\text{number of sera positive in (a) and negative in (b)}}{\text{total number of sera investigated}} \times 100$$

*Identical means either positive or negative

For statistical analysis, the χ^2 test for randomised block designs with data arranged in two classes was used.

Results

When samples arrived in the Netherlands, 308 of 969 samples contained too little serum for carrying out the RPR, TPHA, TPI, and FTA-ABS tests; these samples were, therefore, excluded from this study.

Of the remaining 661 serum samples, the VDRL test gave positive results in 131; of these 131 patients, seven had primary yaws, eight secondary yaws, and 116 latent yaws.

Table 1 shows the reactivity of the various serological tests on the 661 samples. The FTA-ABS test was reactive for 261 samples; for 154 of these the result was positive and for 107 weakly positive. For these 107 cases, the FTA-ABS test was the only test giving a reactive result. The 107 patients showed no signs or symptoms and gave no history of treponemal disease. All tests were reactive for the 15 cases of early infectious yaws.

In Table 2 the results of the FTA-ABS and TPI tests are compared. The agreement between the

Table 1 Reactivity of various tests on sera from 661 children from a region of hypoendemic yaws infection

Test	Sera with positive results		No. of sera with negative results
	No.	%	
VDRL	131	19.8	530
RPR	127	19.2	534
TPHA	139	21.0	522
TPI	147	22.1	514
FTA-ABS	261*	39.5	400

*107 sera with weakly positive results included

Table 2 Comparison of results of FTA-ABS and TPI tests

TPI test results	FTA-ABS test results		
	Negative	Positive*	Total
Negative	396	118	514
Positive	4	143	147
Total	400	261	661

*107 weakly positive results to FTA-ABS tests (all negative in TPI test) included

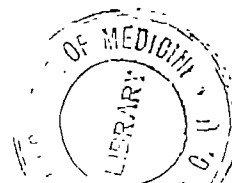
results of FTA-ABS test and TPI test, calculated from the figures presented in this Table, is 81.5%. If the 107 sera with a weakly positive result in the FTA-ABS test are not regarded as giving positive results, the agreement between the FTA-ABS and TPI tests is 97.3%.

The results of the VDRL, RPR, and TPHA tests (screening tests), when the TPI test is used as a reference, are compared in Table 3. There appear to be no statistically significant differences between the three screening tests when agreement, sensitivity, and rate of possible false-positive reactions are taken as criteria. If the FTA-ABS test is used as the reference test instead of the TPI test (Table 2) again no statistically significant differences are found between the three screening tests (level of significance, 5%).

Table 3 Comparison of results of VDRL, RPR, and TPHA test on 661 sera when either the TPI test or the FTA-ABS test* was used as reference. (Figures for the FTA-ABS test used as a reference are given in brackets)

	VDRL test (%)	RPR test (%)	TPHA test (%)
Agreement	93.0 (94.1)	94.3 (95.0)	94.6 (96.2)
Sensitivity	79.6 (79.9)	80.2 (80.5)	85.0 (87.0)
Rate of possible false-positive reactions	2.1 (1.2)	1.4 (0.5)	2.1 (0.8)

*In these calculations only the results of sera showing an intensity of fluorescence scored as ++ or more in the FTA-ABS test are considered as reactive



Discussion

A single, weakly reactive FTA-ABS result in the absence of a history and clinical signs or symptoms of treponemal disease was found for 107 (16.2%) of 661 sera. Garner *et al.* (1970) reported a similar finding for 4.6% of 848 sera from an area of yaws infection. In 10 of our cases new serum samples were collected after one year and the FTA-ABS test was still weakly positive and was the only test showing reactivity.

This indicates that a single, weakly reactive result to the FTA-ABS test is not necessarily the first sign of an infection with pathogenic treponemes. The cause of a single, weakly reactive result is not known; several hypotheses, however, can be put forward:

- (1) Individuals with a single, weakly reactive result to the FTA-ABS test may have been infected by pathogenic treponemes; they may not, however, have developed clinical signs of disease nor an extensive humoral immunological response.
- (2) Not all non-specific antibodies may have been removed from the serum with our ultrasonicate. The weak fluorescence might have disappeared if an ultrasonicate from other non-pathogenic treponemes as well as Reiter treponemes had been used.
- (3) The single, weakly reactive result to the FTA-ABS test may have been caused by other (non-treponemal) diseases and should be considered as a biological false-positive reaction.

Whatever the cause, the single, weakly reactive result does not indicate active treponemal disease and seems to have few practical consequences.

The TPI test is generally accepted as a test for confirmation of treponemal disease. If only those sera which gave results of ++ or more in the FTA-ABS test are considered as reactive, the agreement between the FTA-ABS test and the TPI test appears to be 97.3%. Thus, the FTA-ABS

test can also be used as a confirmatory test for yaws, if only the results of sera showing an intensity of fluorescence scored as ++ or more are considered as reactive.

There appear to be no significant differences between the VDRL, RPR, and TPHA tests—all of which are generally recommended for screening—when agreement with either TPI or FTA-ABS tests, sensitivity, and rate of possible false-positive reactions are taken as criteria. Thus, other criteria, such as cost and labour, should be taken into account when one of these screening tests is selected for future use.

Although the figures in Table 3 suggest that the TPHA test is more sensitive than the other two screening tests, the differences in sensitivity are not statistically significant. According to MacFarlane and Elias-Jones (1977), the sensitivity of the Wellcome TPHA test appears to increase if this test is performed at a lower screening dilution than recommended by the manufacturer. Our results, therefore, might have been different if the TPHA test had been performed as suggested by these authors.

We thank Mr A. B. Leussink of the Department of Biometrical Analysis, the National Institute of Public Health, Bilthoven, for his help. TPHA-test reagents were kindly supplied by Wellcome Nederland BV, Amsterdam.

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Double-staining procedure for the fluorescent treponemal antibody absorption (FTA-ABS) test

ELIZABETH F. HUNTER, ROGER M. MCKINNEY, SHIRLEY E. MADDISON,
AND DONNA D. CRUCE

From the Center for Disease Control, Atlanta, Georgia, USA

SUMMARY The fluorescent treponemal antibody absorption (FTA-ABS) double-staining procedure was reproducible, comparable to the conventional test, and easy to read. We recommend the use of the FTA-ABS double-staining procedure for microscopes with incident illumination, the 100 $\times$ /1.30 oil achromatic objective and the 6.3 $\times$ ocular to obtain optimal fluorescence, and the KP560 as a barrier filter to exclude rhodamine emission when fluorescein fluorescence is read. With this system, errors related to poor focusing or failure to visualise treponemes on all smears should be eliminated.

Introduction

A double-staining technique has been developed for use in the fluorescent treponemal antibody absorption (FTA-ABS) test (National Communicable Disease Center, 1969). This procedure allows the new improved microscopy equipment to be used, specifically the incident illumination microscope. In the incident illumination system described by Ploem (1967) dichroic mirrors placed in the microscope tube reflect the exciting light through the objective; the objective then serves as the condenser. This system eliminates the necessity for proper alignment and focusing of the conventional darkfield condenser, and the microscope is much easier to use. The equipment offers a number of advantages and is especially useful for quantitative fluorescence measurements. For the present FTA-ABS test, however, unless the microscope is also equipped for transmitted darkfield illumination (thereby increasing cost), the reader is unable to verify the presence of treponemes on non-reactive slides. It is to solve the latter problem that our attention was directed to the incident illumination microscopes, which have become increasingly popular.

Materials and methods

Treponema pallidum antigen and sorbent were the reference reagents used for the conventional FTA-ABS test with transmitted illumination and were

obtained through the Biological Products Division, Center for Disease Control (CDC), Atlanta, Georgia. Sera for preparation of controls and evaluation of the test were characterised samples from syphilitic and non-syphilitic subjects obtained through the Venereal Disease Serum Bank, CDC, or were fresh sera from a public health service outpatient clinic.

The primary antibody was goat anti-Fc fragment of pooled normal human IgG. The secondary antibody was IgG syphilitic serum obtained from sera of patients with clinically documented untreated, latent, or untreated secondary syphilis. The IgG of both sera was obtained by DEAE Sephadex chromatography with 0.1 mol/l NaCl containing 0.05 mol/l boric acid buffer adjusted to pH 8.0 with NaOH. The primary and secondary globulins were labelled with either fluorescein isothiocyanate or tetramethyl rhodamine isothiocyanate according to procedures described by McKinney *et al.* (1976). Additionally, a class-specific fluorescein labelled anti-human IgG reagent was obtained from Burroughs Wellcome Inc. Specificity for IgG was established as described earlier (Reimer *et al.*, 1970; Hunter *et al.*, 1972; Hunter *et al.*, 1976).

DOUBLE-STAINING PROCEDURE

Two methods of double-staining were examined.

Method A

The procedure is essentially the same as the conventional FTA-ABS test (National Communicable Disease Center, 1969). Absorbed human serum was incubated with *T. pallidum* antigen in the first step. The preparation was washed, and a fluorescein-labelled anti-human IgG globulin was added to

Address for reprints: Dr E. F. Hunter, Center for Disease Control
Atlanta, Georgia 30333, USA

Received for publication 29 August 1978

demonstrate syphilitic antibody attachment. Slides were rinsed in phosphate-buffered saline. Then, in an additional step, the smears were stained with rhodamine-labelled human anti-*T. pallidum* globulin. In this step all treponemes are stained; therefore, with incident illumination, reactive and non-reactive treponemes could be visualised without a darkfield condenser.

Method B

The procedure is the same as that for method A, except that the label of the two globulins was reversed. The primary reagent was rhodamine-labelled anti-human IgG, and fluorescein-labelled anti-*T. pallidum* globulin was used in the final step.

MICROSCOPE

The Leitz Ortholux II microscope we used was equipped with the Ploemopak multi-wavelength fluorescence illuminator and a high pressure mercury arc lamp (HBO-200). The basic filter system for fluorescein consisted of the BG-38 red-suppressing filter, the K480 edge filter, two KP490 exciter filters, the TK510 dichroic beam splitter, and the K515 barrier filter. Additionally, a KP560 filter was inserted on the slider as a barrier filter to exclude rhodamine emission (Haaijman, 1977). The filters for rhodamine excitation included the red-suppressing BG-38, the BG-36 band-absorption glass filter to absorb the strong mercury line at 578 nm, the KP560 as the exciter filter, the K530 edge filter, the TK580 dichroic beam splitter, and the K590 barrier filter. The optical equipment included 10× and 6.3× oculars, a fluorite (F1) 40×/1.30 oil, and a 100×/1.30 oil achromatic objective.

Results and discussion

When two fluorescent dyes are present in the same preparation the fluorescence emission must be filtered to allow the observer to visualise both dyes selectively. With the fluorescein and rhodamine dyes excitation of fluorescein will result in some excitation of rhodamine. Without a selective emission filter, staining results may be interpreted incorrectly (Haaijman, 1977). In the figure we show the emission spectra for fluorescein- and rhodamine-labelled conjugates. By adding a KP560 as a barrier filter we are able to observe fluorescein staining, and rhodamine staining is completely excluded.

Traditionally, the FTA-ABS test has been read with a 10× ocular and a 40× dry objective. We attempted to read the double-stained slides with a 10× ocular and a F1 40×/1.30 oil objective, but

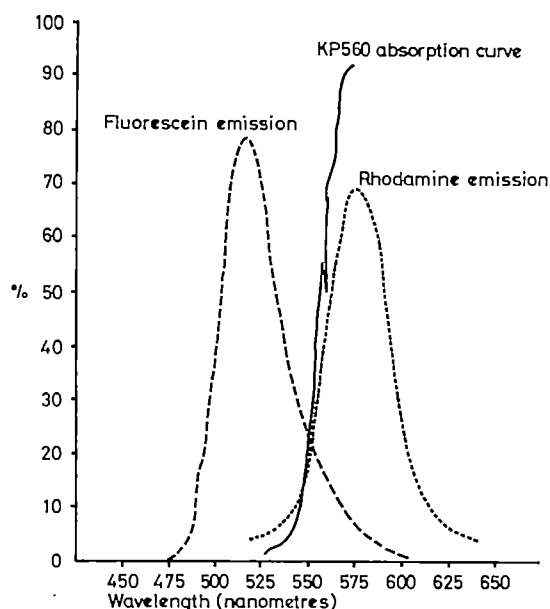


Figure Emission spectra for fluorescein and rhodamine conjugates and KP560 absorption curve

image quality was poor and there was excessive background when non-reactive slides were read with the fluorescein filters (Table 1). The fluorescein filters without the KP560 barrier should pass sufficient rhodamine emission for the 4+ rhodamine counterstaining on non-reactive treponemes to be visualised as brick-red in colour. This would offer

Table 1 Quality of FTA-ABS test results read by incident illumination on a Leitz Ortholux II microscope equipped with a 10× ocular and a F1 40×/1.30 oil objective (500×)

<i>Microscopical evaluation</i>			
<i>Filter system</i>	<i>Fluorescence</i>	<i>Image quality</i>	<i>Background</i>
Fluorescein BG38 K480 2KP490 TK510 K515	S	U	U
Above filters + KP560	S	S	S
Rhodamine BG38 BG36 KP560 K530 TK580 K590	S	S	S

S Satisfactory
U Unsatisfactory

another possibility for observing treponemes on non-reactive FTA-ABS test smears without the added cost of the multiple Ploem filter system. These optics were considered unsatisfactory for this purpose perhaps because of spherical aberration of the objective and eyepiece magnification (Haaijman, 1977). All other readings were satisfactory.

For the FTA-ABS double-staining procedure a $100\times/1.30$ oil achromatic objective with a $6.3\times$ ocular is recommended. We obtained excellent fluorescence, excellent image quality, and an excellent background (Table 2). With this combination non-reactive treponemes could be seen with the single fluorescein filter system because image quality and background were much improved. The brick-red colour was not as easy to read as the maximum red fluorescence obtained with the rhodamine filter system, but the system was satisfactory.

The addition of rhodamine-labelled anti-*T. pallidum* globulin in the final step of method A stained all treponemes red. We recommend, therefore, that treponemes be located and focused with the rhodamine filter system. The $40\times$ oil or $40\times$ dry objective may be easier to use in finding the field. After the field is focused, the $100\times$ oil objective is moved in place. Then, with the selector knob available on the Ploemopak the fluorescein filters are switched in position, the KP560 is inserted, and fluorescein fluorescence is correctly read. After fluorescence intensity has been recorded, slides can be interchanged with very little focus adjustment.

Table 2 Quality of FTA-ABS test results read by incident illumination on a Leitz Ortholux II microscope equipped with a $6.3\times$ ocular and a $100\times/1.3$ oil achromatic objective ($800\times$)

Filter system	Microscopical evaluation		
	Fluorescence	Image quality	Background
Fluorescein BG38 K480 2KP490 TK510 K515	E	E	E
Above filters +KP560	E	E	E
Rhodamine BG38 BG36 KP560 K530 TK580 K590	E	E	E

E Excellent

The slides are much easier to interchange and focus with this equipment than with the darkfield condenser.

We prepared reading controls for the double-staining procedure and evaluated the test with FTA-ABS test results obtained by reading with transmitted light. We obtained essentially the same results as were obtained in the conventional test on sera from 92 syphilitic subjects (Table 3). The differences were between a borderline and a non-reactive and a borderline and a reactive result. Results of tests on sera from 108 non-syphilitic subjects were the same (Table 4).

In the early part of our study we chose method A for several reasons: (1) individuals were trained for reading fluorescein fluorescence; (2) individuals may already have an incident illumination microscope with only the fluorescein filter system; and (3) the Ploemopak was expensive. Now that less expensive microscopes accept up to three filter modules we have to reconsider our choice. We examined method B with a class-specific rhodamine-labelled

Table 3 Comparison of results obtained with the FTA-ABS double-staining procedure and with the conventional test of sera from 92 syphilitic subjects

Category of syphilis	FTA-ABS test					
	Double-staining test			Conventional test		
	R	B	N	R	B	N
Primary	30		4	30	1	3
Secondary	26			26		
Latent	31	1		32		
Total	87	1	4	88	1	3

R Reactive
B Borderline
N Non-reactive

Table 4 Comparison of results of the FTA-ABS double-staining procedure and the conventional test with sera from 108 non-syphilitic subjects

Category	FTA-ABS test					
	Double-staining test			Conventional test		
	R	B	N	R	B	N
Presumed normals			70			70
Diseases other than syphilis			18			18
Biological false-positive			20			20
Total			108			108

R Reactive
B Borderline
N Non-reactive

anti-IgG conjugate and a fluorescein-labelled anti-*T. pallidum* globulin. This appears to be a workable system, but further evaluation is needed.

Conclusion

We recommend use of the double-staining procedure in laboratories where microscopes with incident illumination are available. The FTA-ABS double-staining procedure described here was highly satisfactory and easy to perform. We do not recommend the use of these microscopes with the single-staining FTA-ABS procedure (National Communicable Disease Center, 1969).

Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or by the US Department of Health, Education, and Welfare.

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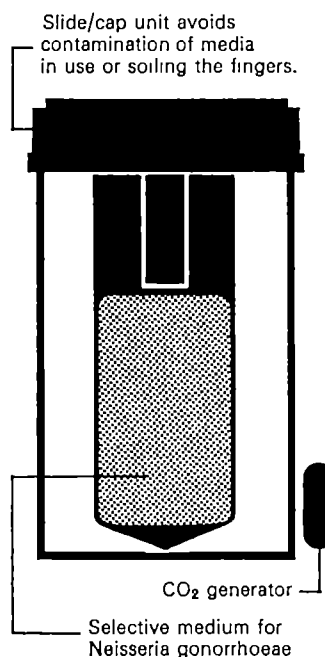
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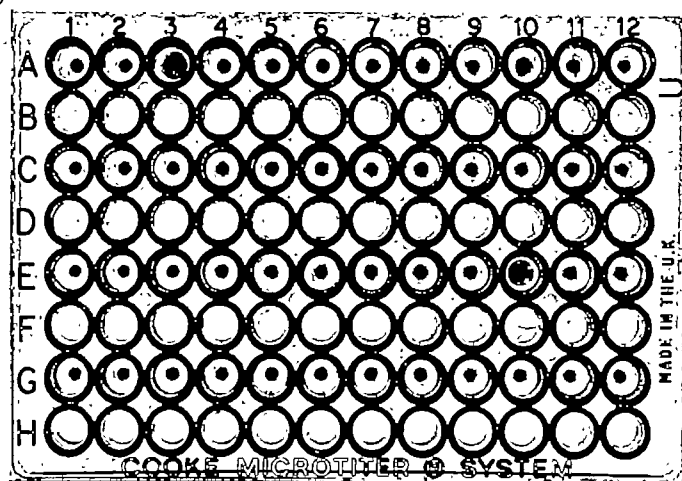


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The HA test would seem to be a practical compromise between the highly sensitive but technically complex RIA test and other simple but less sensitive tests".

Lancet (1974) i 949

A note on microscopy

F. J. AUMONIER

From St Mary's Hospital, London

The path of light rays through a typical vertical illuminator and the Ploemopak illuminator are shown in the Figures (Figs. 1 and 2; by courtesy of Messrs E. Leitz Ltd). In the latter, which is a development of the system used by metallurgists in the nineteenth century, the field diaphragm controls the size of the illuminated field. The exciter and barrier filters are available in four sets, which give a choice of different exciting and barrier filters which can be interchanged in a rotating turret. The beam splitter is also a dichroic filter which totally reflects the exciting wavelength but freely transmits the fluorescent light.

Address for reprints: Dr F. J. Aumonier, St Mary's Hospital, London W2

Received for publication 12 September 1978

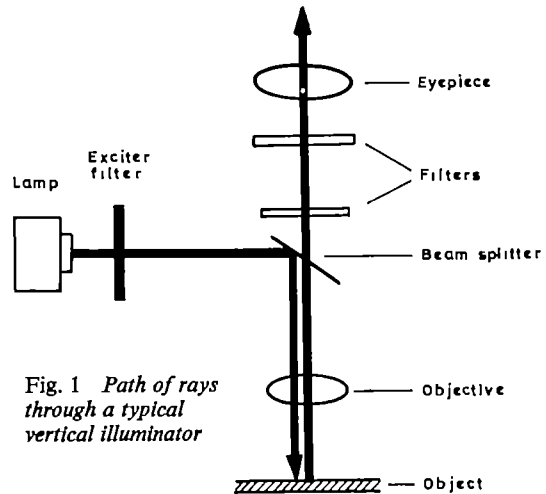


Fig. 1 Path of rays through a typical vertical illuminator

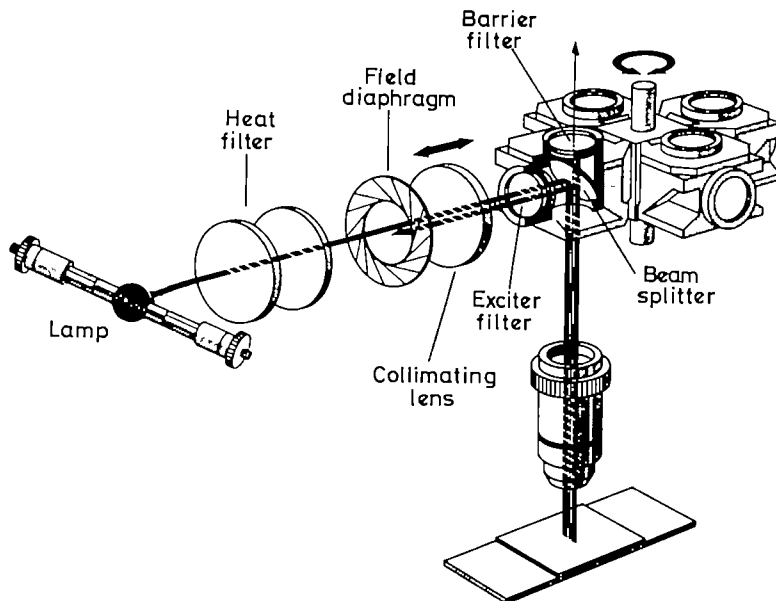


Fig. 2 Path of rays through the Ploemopak illuminator

Therapeutic effect of oral doxycycline on syphilis

YOICHI ONODA

From the Tokyo Metropolitan Taito Hospital, and the Kyorin University School of Medicine, Tokyo, Japan

SUMMARY Fifty-one patients with syphilis were treated with oral doxycycline. A course of the antibiotic treatment consisted of 200 mg of doxycycline daily in two divided doses for 28 days. The courses were repeated three to four times a year with an interval of several months. Quantitative Venereal Disease Research Laboratory (VDRL), Wassermann reaction (WR), and *Treponema pallidum* haemagglutination assay (TPHA) tests were performed monthly to evaluate the therapeutic effect of doxycycline treatment. The response rate was 100% for primary, 90% for early, 68% for late, and 90% for congenital syphilis in adults. No notable side effects were encountered except for epigastric fullness in one patient, which did not require the treatment to be discontinued. No abnormalities were detected in the results of laboratory tests.

Introduction

When given orally in the treatment of syphilis conventional antibiotics should be administered every six hours because of the short duration of their effective blood concentrations. Since anti-syphilitic treatments usually need to be carried out over long periods of time, patients may not strictly follow the treatment regimen.

Although the tetracycline group of antibiotics, including tetracycline itself and oxytetracycline, is known to have a treponemicidal action, these drugs should be given every six hours by mouth to maintain an effective blood concentration. The usual daily dose is 1 g in adults. The continuous use of tetracycline and oxytetracycline over long periods of time may cause a deposit of yellow pigment in the bone. Yellowish discoloration may develop in the teeth of children whose mothers have been given these tetracyclines during pregnancy. Other side effects such as a black tongue also occur in a few cases relatively early in the treatment and leads to discontinuing the administration. Accordingly, tetracycline and oxytetracycline are not satisfactory oral antibiotics in the treatment of syphilis.

The effective blood concentration of doxycycline (DOTC), a derivative of tetracycline, lasts for a period of 12 hours or longer after an oral dose of 100 mg. The daily dose of 200 mg (in two equal

doses) of DOTC, therefore, is one-fifth of that of conventional tetracyclines and will rarely cause any side effects; furthermore, two doses daily are more convenient for patients than four doses daily.

This paper describes the results of DOTC therapy for syphilis over a period of eight years.

Material and methods

PATIENTS

DOTC therapy was given to 51 patients with various stages of syphilis. Thirty-five patients with primary, early, or adult congenital syphilis had had no previous treatment, while seven of 16 patients with late latent syphilis had previously been given some antisyphilitic treatment.

DOSAGE AND ADMINISTRATION OF DOXYCYCLINE

A dose of 100 mg DOTC was given orally, immediately after the morning and evening meal. A course of DOTC treatment consisted of daily administration at this dosage over a period of four weeks (28 days) and was repeated every three to four months. Accordingly, patients received three to four courses of the treatment annually.

LABORATORY TESTS

Quantitative Wassermann reaction (WR), Venereal Disease Research Laboratory (VDRL), and *Treponema pallidum* haemagglutination (TPHA) tests were repeated once a month during the observation period in each case. Liver function was checked by

Address for reprints: Dr Y. Onoda, Tokyo Metropolitan Taito Hospital, 5-20-3 Senzoku Taitoku, Tokyo III, Japan.

Received for publication 7 September 1978

repeat determinations of serum aspartate transaminase (SGOT) and serum alanine aminotransferase (SGPT) levels at appropriate intervals.

OBSERVATION PERIOD

Observation periods ranged between two and nine months for five cases with primary syphilis; this period was short because the disease required only short periods of time for cure. Ten cases with early syphilis were followed over periods up to five years and seven months, 16 cases with late latent syphilis for up to four years and ten months, and 20 cases with adult congenital syphilis for up to seven years and nine months. Physical examination and laboratory tests were repeated every month in each case during the observation period.

CRITERIA FOR RESPONSE TO TREATMENT

The result of DOTC therapy was evaluated by comparing titres of the VDRL and WR tests before and after treatment. The response was considered excellent when titres of both tests decreased by more than two dilutions and when the titre of one test was decreased by one dilution or not at all but that of the other test was decreased by more than two dilutions. A good response was so defined when the titre of one test was decreased by two dilutions but the titre of the other test was decreased by one dilution or remained unchanged. The response was evaluated as poor when only a one dilution difference was observed in the titre of one test before and after DOTC therapy but none in the titre of the other test, or no difference was found in the titre of either test before and after treatment (Table 1).

Results

PRIMARY SYPHILIS

Laboratory data for a patient with primary syphilis whose antibody titres were decreasing to zero at short intervals after DOTC therapy are shown in Table 2.

Table 1 *Criteria for response to oral doxycycline therapy*

Response	Decrease in antibody titre (dilutions)*	
	VDRL	WR
Excellent	2	2
	2	1
	2	0
Good	2	1
	2	0
	1	1
Poor	1	0
	0	0

*Difference in dilutions before and after treatment

The results of the serological tests for syphilis reverted to normal in each of five patients with primary syphilis at the end of DOTC therapy, which ranged in duration from two to nine months. Thus, the response was excellent in all of the five cases (Table 3).

SECONDARY SYPHILIS

The course of a case of early syphilis is shown in Table 4; an excellent response to DOTC therapy was seen during the observation period of one year and nine months.

Ten patients with secondary syphilis were treated with the same regimen. The shortest observation period was four months in four patients, while one patient was followed up for a period as long as five years and seven months (Table 5). The response to DOTC therapy was excellent in five patients, good in four, and poor in the remaining one. The patient with a poor response has been followed up for a period of only four months. Since excellent responses were achieved in two patients followed up for comparable periods of time, however, an evaluation of poor response was made in this patient.

LATE SYPHILIS

In one patient alopecia had developed due to a syphilitic infection which had occurred 10 years previously and was unnoticed and untreated (Table 6). Oral DOTC therapy consisting of 12 courses during a period of four years and four months produced an excellent response in this patient.

Of 16 cases of late syphilis nine had had no previous treatment while seven had previously received arsenicals or bismuth preparations. Of 16 cases with late syphilis, six (37.5%) responded excellently, five (31.3%) well, and five (31.3%) poorly to the treatment. Of nine cases previously untreated, five (55.6%) showed excellent responses, and four (44.4%) good responses (Table 7).

ADULT CONGENITAL SYPHILIS

A female patient who had positive results to serological tests at a premarital examination responded excellently to oral DOTC therapy. She received 21 courses during a period of seven years and nine months (Table 8).

Of 20 patients with adult congenital syphilis who had had no previous treatment, 12 (60.0%) showed excellent responses, six (30.0%) good responses, and two (10.0%) poor responses (Table 9).

SIDE EFFECTS

Epigastric fullness occurred in some patients, but it was only transient. No patient had to stop treatment because of gastrointestinal disturbances.

Table 2 *Laboratory data for a 36-year-old male patient with primary syphilis*

Date of examination	Observation period (months)	Antibody titres			Course of DOTC therapy*
		VDRL	WR	TPHA	
October 1972	0	16	64	320	1st
November 1972	1	4	16	320	
February 1973	4	0	0	320	2nd
March 1973	5	0	0	320	
July 1973	9	0	0	320	

*Each course consisted of daily doses of 200 mg for 28 days

Table 3 *Laboratory data for five patients with primary syphilis*

Case no.	Age (yrs)	Sex	Antibody titres						Observa- tion period (months)	No. of courses of DOTC therapy	Response
			Before treatment			After treatment					
			VDRL	WR	TPHA	VDRL	WR	TPHA			
1	36	M	16	64	320	0	0	320	9	2	Excellent
2	29	M	4	16	0	0	0	0	6	1	Excellent
3	32	M	2	40	1280	0	0	1280	4	1	Excellent
4	41	M	32	1024	5120	4	64	5120	3	1	Excellent
5	26	M	1	0	0	0	0	0	2	1	Excellent

Table 4 *Laboratory data for a 56-year-old male patient with early syphilis*

Date	Observation period (months)	Antibody titres			Course of DOTC therapy*
		VDRL	WR	TPHA	
August 1975	0	32	128	5120	First
September 1975	1	32	256	5120	
October 1975	2	32	256	5120	Second
December 1975	4	32	128	1280	
January 1976	5	8	64	5120	Thrd
February 1976	6	8	32	5120	
April 1976	8	8	64	1280	
November 1976	15	4	16	5120	Fifth
January 1977	17	2	8	1280	
March 1977	21	2	16	1280	

*Each course consisted of 200 mg daily for 28 days

Table 5 *Laboratory data for 10 patients with early syphilis*

Case no.	Age (yrs)	Sex	Antibody titres						Observation period (months)	No. of courses of DOTC therapy	Response
			Before treatment			After treatment					
			VDRL	WR	TPHA	VDRL	WR	TPHA			
1	33	M	64	1024	5120	2	8	1280	4	1	Excellent
2	42	F	64	256	5120	32	128	1280	4	1	Good
3	28	F	32	256	> 5120	4	32	1280	4	1	Excellent
4	25	F	2	32	5120	2	32	5120	4	1	Poor
5	25	M	128	1024	1280	4	64	5120	6	2	Excellent
6	23	M	32	128	1280	8	256	320	8	2	Good
7	45	M	32	128	5120	8	64	5120	13	2	Good
8	56	M	32	128	5120	2	16	1280	19	5	Excellent
9	24	M	64	256	5120	16	128	1280	45	7	Good
10	33	M	64	800	5120	1	0	320	67	1	Excellent

Table 6 Laboratory data for a 33-year-old male patient with late latent syphilis who had had alopecia due to a previous infection

Date of examination	Observation period	Antibody titres			Course of DOTC therapy*
		VDRL	WR	TPHA	
February 1973	0	128	256	5120	First
March 1973	2	64	256	5120	Second
September 1973	7	8	8	5120	Third
December 1973	10	8	32	5120	Fourth
May 1974	15	4	32	1280	Fifth
December 1974	22	2	16	5120	Sixth
April 1975	26	8	32	1280	Seventh
August 1975	30	8	32	1280	Eighth
January 1976	35	2	8	1280	Ninth
July 1976	41	2	16	1280	Tenth
November 1976	45	2	8	1280	Eleventh
April 1977	50	1	8	320	Twelfth
June 1977	52	1	8	1280	

*Each course consisted of 200 mg doses daily for 28 days

Table 7 Laboratory data for 16 patients with late syphilis

Case no.*	Age (yrs)	Sex	Antibody titres						Observation period	No. of courses of DOTC therapy	Response
			Before treatment			After treatment					
			VDRL	WR	TPHA	VDRL	WR	TPHA			
(1)	37	F	1	0	80	0	0	80	4	1	Excellent
(2)	65	M	4	16	1280	2	8	1280	4	1	Good
(3)	55	M	2	4	320	2	32	320	13	3	Poor
4	63	M	2	32	1280	2	8	1280	15	3	Good
5	33	F	1	16	320	1	4	320	16	4	Good
6	29	M	4	8	320	2	4	320	16	4	Good
7	52	M	1	8	320	2	8	320	16	2	Poor
8	55	M	2	8	320	1	0	80	23	4	Excellent
9	38	M	16	160	320	4	8	320	26	7	Excellent
(10)	65	F	128	1024	5120	64	512	5120	26	7	Good
(11)	52	M	32	256	5120	32	128	5120	26	4	Poor
(12)	45	F	8	16	320	4	16	5120	40	7	Poor
13	51	F	8	32	320	2	8	1280	48	10	Excellent
14	33	M	128	256	5120	1	8	1280	52	12	Excellent
15	49	M	2	16	1280	2	0	320	58	7	Excellent
16	31	M	8	32	1280	2	16	5120	58	12	Good

*Case nos. in parenthesis indicate those having had previous treatment

Table 8 Laboratory data for a 23-year-old female patient with adult congenital syphilis

Date	Observation period	Antibody titres			Course of DOTC therapy
		VDRL	WR	TPHA	
February 1970	0	16	128	81920	First
November 1970	9	32	128	20480	Third
May 1971	15	8	128	5120	Fourth
November 1971	21	16	32	5120	Fifth
May 1972	27	8	64	5120	Sixth
October 1972	32	16	64	5120	Seventh
March 1973	37	16	128	5120	Eighth
July 1973	41	8	32	5120	Ninth
February 1974	48	8	32	5120	Eleventh
November 1974	57	16	64	5120	Thirteenth
March 1975	61	8	64	5120	Fourteenth
November 1975	69	8	32	5120	Sixteenth
March 1976	73	8	64	5120	Seventeenth
November 1976	81	8	32	5120	Nineteenth
March 1977	85	8	64	5120	Twentieth
July 1977	89	8	128	5120	Twenty-first
September 1977	91	4	16	5120	—
November 1977	93	4	32	5120	—

Table 9 Laboratory data for 20 patients with adult congenital syphilis

Case no.	Age (yrs)	Sex	Antibody titres						Observation period	No. of courses of DOTC therapy	Response
			Before treatment			After treatment					
			VDRL	WR	TPHA	VDRL	WR	TPHA			
1	57	M	1	8	320	0	0	5120	6	2	Excellent
2	63	M	16	128	1280	8	64	5120	12	4	Good
3	42	F	16	64	5120	8	32	5120	14	4	Good
4	45	F	2	8	320	1	0	80	18	8	Excellent
5	36	F	4	64	320	1	16	80	21	2	Excellent
6	28	M	8	128	5120	2	8	1280	24	3	Excellent
7	30	M	2	4	1280	1	16	1280	34	8	Poor
8	55	F	4	16	320	2	8	320	37	10	Good
9	32	M	32	256	5120	8	16	5120	38	9	Excellent
10	30	M	16	128	5120	8	32	5120	39	9	Good
11	63	F	16	128	320	8	16	80	42	10	Excellent
12	34	M	32	128	5120	1	8	5120	49	10	Excellent
13	48	M	4	16	320	4	16	320	55	12	Poor
14	34	M	32	256	5120	8	64	5120	59	15	Excellent
15	26	F	16	128	320	8	8	320	60	13	Excellent
16	24	M	2	16	5120	2	0	1280	61	9	Excellent
17	29	M	32	256	20480	16	64	5120	63	15	Good
18	27	F	32	256	5120	4	16	1280	85	11	Excellent
19	30	F	2	32	320	1	16	320	89	5	Good
20	23	F	16	128	81920	4	32	5120	93	20	Excellent

SGOT and SGPT levels remained within normal range during the entire period of observation in each case. Herxheimer's phenomenon was not observed even in those patients with primary and secondary syphilis.

Discussion and conclusion

Oral penicillins, cephalosporins, macrolides, and tetracyclines are effective in the treatment of syphilis. Oral administration of these antibiotics is inconvenient since they should be taken every six hours to maintain an effective blood concentration. Unless the regimen is strictly followed, satisfactory results will not be obtained even with the most potent drugs. Oral antibiotics characterised by long-lasting effective blood concentrations are required for the treatment of syphilis where effective blood concentrations should be maintained without interruption over prolonged periods of time.

DOTC was found to satisfy these criteria for the treatment of syphilis. Two daily doses were sufficient

to maintain effective blood concentrations, and such a regimen was convenient for patients. Few side effects developed because daily doses were low. Generally, patients followed the regimen precisely, and changes in antibody titres due to the therapy could be serially observed.

Alexander and Schoch (1966), Steppert (1968), and Wodniansky *et al.* (1969) have reported their short-term observations of patients after treatment with DOTC, and they all noticed excellent results. I have also treated a patient with primary syphilis using DOTC 100 mg daily for 14 consecutive days with excellent results. Tsutogawa *et al.* (1971), Minami *et al.* (1972) and Maruta and Shirouzu (1973) reported good results using the same dose of DOTC for patients with syphilis.

The overall effective rates in this study were 100% for patients with primary syphilis, 90% for those with early syphilis, 68.7% for those with late syphilis (90% for those without previous treatment), and 90% for those with adult congenital syphilis (Table 10).

Table 10 Summary of the effects of oral doxycycline treatment for syphilis

Stage of syphilis	Response and number of patients treated						Total no. of cases
	Excellent		Good		Poor		
	No.	%	No.	%	No.	%	
Primary	5	100.0	0	0	0	0	5
Early	5	50.0	4	40.0	1	10.0	10
Late	6	37.5	6	37.5	4	25.0	16
Adult congenital	12	60.0	6	30.0	2	10.0	20

Onoda *et al.* (1977) treated 61 cases of syphilis with amoxycillin for two years in a previous clinical trial. This oral treatment was effective in 100% of cases of primary and early syphilis, in 66.7% of cases of late syphilis, and in 60% of cases of adult congenital syphilis.

Treatment with both amoxycillin and DOTC proved effective in 100% of cases of primary syphilis. For early syphilis, the effective rate with DOTC therapy was lower than that with amoxycillin therapy, although the difference did not seem to be significant. Also, there was no significant difference between the therapeutic effect of amoxycillin and DOTC on late syphilis. Treatment with DOTC was more effective than with amoxycillin for adult congenital syphilis, probably because of the longer observation periods used for the former therapy than for the latter.

The antisymphilitic effect of oral DOTC was proved to be comparable or somewhat superior to that of oral penicillins, probably because less frequent doses were required with DOTC than with penicillins. The more frequent the daily doses the more easily patients would forget to take the drug. In addition, few side effects were caused by DOTC treatment. Thus, oral DOTC therapy is suitable for the treatment of late syphilis and congenital syphilis in adults, which require treatment over long periods of time.

As a result of the introduction of penicillins the

number of patients with syphilis has been notably reduced. The disease has by no means been eradicated, however, but its incidence has tended to increase in recent years. Injections of penicillins may be contraindicated in occasional cases and, generally, patients prefer oral administration to injection. The development of satisfactory oral antibiotic treatment regimens for syphilis, such as discussed in this paper, are needed.

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Theme 3: Recent advances in the study of STDs due to viral and other agents

Follow up of patients with hepatitis B surface antigen

P. D. SIMMONS

From the Department of Genital Medicine, St Bartholomew's Hospital, London

SUMMARY One hundred and five patients attending clinics for sexually transmitted diseases were found to carry hepatitis B surface antigen (HBsAg). Of these, 87 patients had liver function tests performed of which 44 gave abnormal results. Fifteen patients underwent liver biopsy, which showed chronic hepatitis in eight cases.

Introduction

Much work has been done on the epidemiology of hepatitis B (Zuckerman, 1972), including its probable sexual transmission (Zuckerman, 1977). The medical problems after the hepatitis B surface antigen (HBsAg) carrier has been identified, however, are considerable (*British Medical Journal*, 1974; Woolf *et al.*, 1974). As there is a growing desire among genitourinary physicians to screen for HBsAg carriers, it was decided to determine the incidence of possible liver disease among patients attending sexually transmitted disease (STD) clinics. In the United Kingdom several groups of patients are at greater risk of being carriers of HBsAg (Cossart, 1977); these groups formed the basis for the study.

Material and methods

Patients attending the STD clinics of St Thomas's, St Bartholomew's, and the Prince of Wales's Hospitals, London, were screened for HBsAg, when blood for syphilis antibodies was taken, if they fell within the following groups with a high risk of HBsAg carriage:

- (1) Homosexuals;
- (2) Prostitutes;
- (3) Drug abusers;

- (4) Patients with previous liver disease;
- (5) Tattooed patients;
- (6) Patients born outside the United Kingdom. (This group will include some patients not from particularly high risk areas, but the term is used for ease of interpretation in a busy clinic.)

HBsAg was determined by solid-phase radio-immuno-assay (Abbot Laboratories). HBsAg-positive patients who returned for follow up were questioned closely on further possible factors related to HBsAg carriage, and these are shown in Table 1. Some patients, therefore, had more than one risk factor (Table 2).

Table 1 Other risk factors for HBsAg.

Risk factors	Heterosexuals (74)	Homosexuals (31)
Previous jaundice	5	2
Jaundice at presentation	4	3
Tattooed	1	1
Transfusion/injections	2	2
Pierced ears		1
Nurse		1
Drug abuse	5	1
Born outside UK	65	10

Table 2 Number of risk factors for HBsAg per patient

No. of risk factors	Heterosexuals	Homosexuals
1	66 (98.2%)	18 (58.1%)
2	8	9
3		3
4		1
Total	74	31

Address for reprints: Dr P. D. Simmons, Department of Genital Medicine, St Bartholomew's Hospital, London EC1

Received for publication 19 October 1978

Results

One hundred and five patients who carried HBsAg were identified—that is, 4% of those screened, although the incidence varied according to the hospital the patients attended. Thirty-one male homosexuals were HBsAg-positive, as were 74 heterosexuals—55 male and 19 female; their racial origin is shown in Table 3. Twenty-four (77%) homosexuals came from Europe, including the United Kingdom, and one each from the West Indies, Mauritius, Singapore, United States, India, Australia, and South Africa. Of the heterosexuals, however, only 14 (19%) came from Europe, while 23 (31%) came from the West Indies and 25 (34%) from West Africa (Table 4).

All patients with HBsAg who returned for follow up had liver function tests carried out; these included values for serum bilirubin, protein, albumen, alkaline phosphatase, and aspartate aminotransferase (Table 5). Normal and abnormal results of liver function tests relate to the values set at each of the three hospitals where patients were seen. In only 40% of both homosexuals and heterosexuals was the abnormal value at or above twice the upper limit of the normal value, and many values were only marginally raised. In only two patients was the aspartate aminotransferase not raised when other tests gave abnormal results.

Those patients with abnormal results were referred to a gastroenterologist for further evaluation. Fifteen patients underwent liver biopsy. The results of these biopsies in seven heterosexual patients and eight homosexuals are shown in Table 6. Three homosexual and two heterosexual patients had chronic aggressive hepatitis with

cirrhosis, while two homosexuals and one heterosexual had chronic persistent hepatitis.

Table 5 Liver function test results

	Total no. tested	Normal		Abnormal	
		No.	%	No.	%
Homosexuals	27	7	25.9	20	74.1
Heterosexuals	60	36	60.0	24	40.0

Table 6 Results of liver biopsies in 15 HBsAg carriers

Results of liver biopsy	Heterosexuals	Homosexuals
Normal	2	1
Non-specific inflammation	1	1
Acute hepatitis	1	1
Chronic persistent hepatitis	1	2
Chronic aggressive hepatitis with cirrhosis	2	3
Total	7	8

Discussion

It is clear that isolation of HBsAg from the sera of patients attending STD clinics is only the first step in their investigation. One-third of the patients undergoing liver biopsy had chronic aggressive hepatitis with cirrhosis sufficiently severe to be treated immediately with corticosteroids, while 20% of the patients had chronic persistent hepatitis. All 15 patients who underwent biopsy were completely symptom-free and not abusing drugs or alcohol.

Although homosexuals are at recurrent risk of acquiring HBsAg, and should be regularly screened if no facilities for determining their antibody status are available, this study shows that patients attending STD clinics from high risk areas abroad should also be screened and followed up. Both groups present dangers to laboratory and technical staff and both may have asymptomatic liver disease, which may require urgent treatment and certainly needs advice and observation.

I wish to record my thanks to Dr R. P. H. Thompson at St Thomas's Hospital and Dr M. L. Clark at St Bartholomew's Hospital, London, for their help in assessing some of these patients.

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Table 3 Racial origin of HBsAg-positive patients

Racial groups	Heterosexuals		Homosexuals	
	No.	%	No.	%
Caucasian	16	21.6	28	90.3
Negroid	50	67.6	2	6.5
Mongoloid	3	4.0	1	3.2
Arab	4	5.4		
Asian	1	1.4		
Total	74	100.0	31	100.0

Table 4 Areas of origin (heterosexuals)

Area of origin	No.
UK/Europe	14
West Indies	23
West Africa	25
North Africa	4
Far East	3
South America	3
Kenya	1
Pakistan	1
Total	74

Group B streptococcal infection

A review and update

JOHN M. KNOX

From the Department of Dermatology, Baylor College of Medicine, Houston, Texas, USA

SUMMARY The problems posed by the sudden increase in serious group B streptococcal disease among infants since the early 1970s are described and discussed. Virtually all offspring of colonised mothers harbour the organism and infection develops in about 1% of these infants. The mortality rate, even with immediate antibiotic treatment of early onset disease, is 30%; delay in treatment leads to much higher mortality. Late onset disease starting around the seventh to ninth day of life, but sometimes as late as the second month, is less frequently fatal. Preventive measures include active and passive immunisation or intravenous ampicillin during labour. Experimental evidence indicates that each of these methods gives protection.

Introduction

Sexual activity of the parents may be responsible for the emergence of many cases of serious β -haemolytic group B streptococcal infections among newborn infants. Although β -haemolytic group B streptococcus has been identified as a cause of puerperal and neonatal disease since 1938, the incidence of infection remained low until the late 1960s and early 1970s, when a dramatic increase in serious group B streptococcal infection among infants was noted. Currently, it is predicted that between 12 000 and 15 000 infants in the United States will develop group B streptococcal disease during the next year (Horn *et al.*, 1974). In a number of geographical areas, the group B streptococcus is now the leading cause of meningitis during the first two months of life (Franciosi *et al.*, 1973; Baker *et al.*, 1973). Approximately 50% of affected infants will die; approximately 50% of the survivors with meningeal invasion will develop neurological sequelae (Horn *et al.*, 1974). Because the incidence of serious infection caused by other bacteria has not diminished in recent years, the increase of group B streptococcal disease has resulted in an absolute increase in the number of

cases of serious neonatal bacterial infections in our city (McCracken, 1976).

The reason for the emergence of the group B streptococcus as a cause of serious neonatal infections is unknown. This has prompted numerous investigations concerning colonisation of pregnant women (Franciosi *et al.*, 1973; Baker and Barrett, 1973; Bevanger, 1974; Schauf and Hlaing, 1976; Ferrieri *et al.*, 1977; Aber *et al.*, 1976). Group B streptococci can often be found on normal human mucous membranes. Selective media cultures indicate that from 25% to 35% of asymptomatic pregnant women carry group B streptococci in their vagina. Pharyngeal and anal colonisation are also not uncommon. In approximately 50% of the cases studied, male consorts of colonised women will show recovery of the same serotype streptococcus from their urethra (Eichenwald, 1978).

EARLY ONSET DISEASE

Early onset disease in infants is caused by the same serotype as that which colonises the mother's vaginal tract. This finding indicates that the baby becomes infected either *in utero* or during the birth process. Up to 70% of vertical transmission of group B streptococci from mother to infant, presumably at the time of delivery, has been reported (Baker and Barrett, 1973; Aber *et al.*, 1976). Virtually all offspring of colonised mothers harbour the organisms in one or several sites. Infection develops in about 1% of these infants. Neonatal streptococcal illness may be caused by all

Address for reprints: Professor John M. Knox, Department of Dermatology, Baylor College of Medicine, Texas Medical Center, Houston, Texas 77030, USA

Received for publication 21 November 1978

of the five known serotypes (1A, 1B, 1C, type 2 and type 3) (Eichenwald, 1978).

Early onset streptococcal disease is a critical illness, usually appearing during the first day of life, although symptoms may be delayed for as long as one week. The syndrome is characterised by acute respiratory distress, apnoea, and shock often associated with septicaemia and meningitis. The illness closely resembles hyaline membrane disease, with the lung being the initial and major site of involvement, presumably infected by aspiration of infected vaginal secretions or amniotic fluid. Cultures of the nasopharynx, gastric aspirate, skin, and meconium usually give positive results, as do blood cultures. The ratio of immature to mature polymorphonuclear leucocytes in the peripheral blood may aid in distinguishing group B streptococcal infection from hyaline membrane disease.

Early onset disease is extremely serious; although antimicrobial agents are available for the treatment of group B streptococcal infection, even promptly administered antibiotics are often unable to prevent fatal progression, mortality rates being approximately 30% (Eichenwald, 1978; National Institutes of Health, 1972). When therapy is delayed until laboratory confirmation of group B streptococci is received, most infected infants will die. In our teaching hospitals newborn infants with respiratory distress are treated immediately with antibiotics; these medications are continued until blood cultures have failed to grow the pathogen.

LATE ONSET DISEASE

Late onset group B streptococcal disease is a more limited and less severe type of infection. Symptoms usually begin around the seventh to ninth day of life but may appear as late as the second month. Late onset disease is usually a focal infection and may produce such diverse syndromes as meningitis, cellulitis, arthritis, osteomyelitis, otitis media, ethmoiditis, conjunctivitis, renal infections, purulent pericarditis, and brain abscess (Eichenwald, 1978). Mortality from late onset illness is considerably lower than from early onset disease; however, there is a high incidence of permanent residua. Almost one half of infants presenting with late onset disease are born from non-colonised mothers; furthermore, there is little correlation between the serotype recovered from the infants and that cultured from colonised mothers. These findings indicate that late onset infection is probably acquired by a different mechanism than is early onset disease. Rather than being an expression of a longer incubation period after intrapartum infection, late onset disease is probably acquired from the nursery or at home.

Research at Baylor College of Medicine

Maternal colonisation surveillance continues to be of interest. My associates recently described the prevalence of group B streptococcal colonisation among lower socioeconomic parturients, ascertaining demographic data regarding age, race, marital status, parity, and trimester of pregnancy for 112 women (Beachler *et al.*, 1979). At the time of enrolment in the study, single vaginal and throat swabs were obtained and immediately inoculated into a selective broth medium containing Todd-Hewitt broth (Difco Laboratories), 5% defibrinated sheep red blood cells, gentamicin sulphate 8 µg/ml (Schering Corp.), and nalidixic acid 15 µg/ml (Winthrop Laboratories) (Beachler *et al.*, 1979). The broth cultures were incubated overnight at 35°C and streaked on to sheep blood agar plates, with colonies of non-β-haemolytic and β-haemolytic streptococci being subsequently serogrouped and serotyped by methods described previously (Franciosi, 1973).

Results

Group B streptococci were recovered from 32 (28.6%) of the study participants. Colonisation at vaginal sites alone occurred in 26 subjects (23.4%) and at throat sites alone in five (4.4%). In one patient, streptococci of identical serotype were recovered from both throat and vagina. Thirty-three strains of group B streptococci were isolated from throat and vaginal cultures, with type 3 being the most frequent (39.5%); type 2 (30.3%), 1B (21.2%), 1A (3.0%), and 1C (3.0%) were isolated less frequently. No statistically significant differences were detected between colonised and non-colonised pregnant women with regard to age, race, marital state, or parity.

Ninety-six of the study participants had sera available for detection of antibody directed against the type 3 capsular polysaccharide antigen. Sera were collected at the time of enrolment in the study and stored at -20°C until tested. Serum concentration of antibody was determined by radioactive antigen-binding assay (RABA) (Baker and Kasper, 1976; Baker *et al.*, 1977). Sera from 65 non-colonised control subjects showed a median antibody concentration of 1.00 µg/ml (range 0.376-40.3 µg/ml). Thirty-one women colonised with any of the group B streptococcal serotypes showed a median antibody concentration of 1.12 µg/ml (range 0.442-40.3 µg/ml), and 13 women colonised with type 3 streptococci had a median antibody concentration of 4.84 µg/ml (range 0.550-40.3 µg/ml). Concentration of antibody

to the type 3 capsular polysaccharide in sera from non-colonised subjects was significantly lower than that in sera from women colonised with type 3 streptococci ($P=0.0241$). No statistically significant differences, however, were found in sera from women colonised with the various serotypes of group B streptococci ($P=0.0863$, Mann-Whitney U test) (Snedecor and Cochran, 1967). Frequency distribution of concentration of serum antibody in these 96 parturients studied was similar to that previously reported from pregnant women, neonates, infants, and children; the level of serum antibody may be related in part to increasing age among infants and children (Baker and Kasper, 1976; Baker *et al.*, 1977; Hammerschlag *et al.*, 1977).

Discussion

Little is known concerning the relationship between asymptomatic mucous membrane colonisation with the group B streptococcus in the pregnant woman and the presence of serum antibody to the surface antigens of this bacterium. Recently, Baker and Kasper (1976) demonstrated a significant correlation between very low levels of antibody to the capsular polysaccharide of type 3 group B streptococci in women harbouring type 3 organisms genitally and risk for invasive neonatal disease due to these strains (Baker and Kasper, 1976). Their studies also indicated that low levels of type 3-specific antibody can be detected in sera from women of all ages (Baker *et al.*, 1977; Hammerschlag *et al.*, 1977; Hemming *et al.*, 1976).

Three methods for the prevention of infant colonisation with group B streptococci have been suggested: (1) eradication of vaginal colonisation (and perhaps rectal colonisation) of the pregnant woman by treating her and her spouse with oral penicillin; (2) temporary eradication of vaginal group B streptococci carriage during the intrapartum period by administration of an intravenous penicillin during labour; and (3) penicillin treatment of the infant at the time of birth. The first two of these methods are being investigated by our group. To date, the couple-treatment investigation has yielded disappointing results, as have similar studies by others (Hall *et al.*, 1976). Results of the intravenous antibiotic treatment of women during labour are not complete but appear promising.

If it is correct that antibody-deficient infants are more likely to develop early or late onset disease, two possible immunological approaches are suggested: (1) administration of specific high titre immune globulin to all infants immediately after

birth, which could theoretically prevent late onset disease and perhaps reduce the incidence of early onset disease as well; and (2) development of a polyvalent antistreptococcal vaccine to be administered during pregnancy. Observations in mice with one serotype indicate a considerable degree of protection with each of the above approaches (Eichenwald, 1978). The present lack of any alternative method of prevention, except intravenous ampicillin administered to all mothers harbouring the organism, indicates the need for study of possible methods of immune prophylaxis.

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Vaccination for herpes simplex genitalis

TH. NASEMANN AND S. W. WASSILEW

From the Department of Dermatology, University of Hamburg, Hamburg, Germany

SUMMARY Type-specific vaccines containing heat-inactivated herpes simplex hominis virus type 1 or 2 have been developed for the treatment of herpes simplex genitalis. When patients with recurrent herpes genitalis were treated with the type 2 vaccine the clinical course of the disease improved and the eruption-free interval was extended. In severe cases, especially in permanent genital herpes, the concurrent administration of vaccine and immunoglobulin concentrates improved the clinical course of the disease. Antibody titres and the *in-vitro* stimulation of lymphocytes with phytohaemagglutinin or herpes simplex hominis virus antigens remained constant during administration of the vaccine.

Introduction

Alterations in the cell-mediated immunity of patients with recurrent genital herpes have been observed by several authors using different methods. The studies (Lopez and O'Reilly, 1977; O'Reilly *et al.*, 1977; Wassilew and Koch, 1978) indicate a persistent or temporarily diminished immune response in patients with severe and frequently recurring genital herpes.

The mechanism that diminishes the immune response in these patients is unknown and we do not know if the diminished immune response to herpes simplex virus (HSV) antigens is the cause or an effect of the recurrence.

Findings in a rabbit model suggest that viral shedding can lead to a suppression or depletion of the function of T-lymphocytes (Cappel, 1976). If such is the case, efforts to augment the functional capacity of this population of lymphocytes might serve to increase the host's capacity to restrict viral proliferation and thereby reduce the frequency and the clinical manifestations of the infection.

Type-specific vaccines

Two type-specific vaccines have been developed in Germany; these contain heat-inactivated type 1 (Lupidon H®) or type 2 (Lupidon G®) herpes simplex hominis virus cultured aerobically on chick chorioallantoic membrane.

*Hermal Chemie Kurt Herrmann, 2057 Reinbek, Germany

Address for reprints: Th. Nasemann, Universitäts-Krankenhaus, Eppendorf Martinstr. 52, 2000 Hamburg 20-05, West Germany

Received for publication 5 July 1978

The dosage and period of administration of the vaccine have to be adjusted to suit individual patients. A standard regimen for vaccination with the HSV type 2 antigen is a 1-ml dose containing 10⁴ EID₅₀ (Wassilew and Koch, 1978); the dosage can be increased in severe cases up to 4 ml. The period of administration of the vaccine in many patients has to be extended to two to three years (Table).

SIDE EFFECTS

Up to now, no severe side effects of the vaccine therapy have been reported. In about 3% of patients local swelling has been observed. For theoretical reasons, the oncogenicity of the vaccine has been tested; no direct oncogenic effect with the HSV type 2 vaccine was found (Rapp, personal communication).

Results

Since 1971, the type 2 vaccine has been in clinical use and the results are encouraging. When patients have been treated for extended periods, there is clinical improvement in 60% to 100% of cases.

Table Vaccination regimen for herpes simplex genitalis with herpes simplex virus type 2 antigen (Lupidon G®)

Regimen (months)	Dosage	Frequency
First to third	1 ml	Each week
Fourth and fifth	1 ml	Every second week
Sixth to ninth	1 ml	Each month
For years	1 ml	Every second or third month

Raab summarised the results of different authors and found a mean improvement rate as high as 80% (Koinis and Wüthrich, 1975; Raab, 1977). A double-blind controlled study showed that the placebo without viral antigens brought no significant improvement (Weitgasser, 1977).

In 30 selected patients with immunoglobulin defects, mainly decreased IgG levels, Nasemann (1976) found that the concurrent administration of vaccine and immunoglobulin concentrates as adjuvants improved the clinical results. Of patients with herpes simplex type 2 infections which showed no improvement after vaccination alone, 62% reported improvement after treatment with vaccine and immunoglobulins combined. In double infections with both types of the virus, the improvement rate was lower. Especially in permanent (that is, fresh lesions arising before previous ones had healed) genital herpes, the combined therapy seemed to be indicated (Nasemann, 1976; Pra, 1977).

Clinical improvement is difficult to define in recurrent genital herpes. The natural course of the disease is unpredictable. We observed the patients weekly for three acute episodes before starting vaccination and for six months after completing vaccination. In our studies, we regarded a herpes-free interval after therapy which was twice as long as the interval before therapy as an improvement. Because clinical improvement is not objective, it seems essential to measure immunological parameters during vaccine therapy.

Remy *et al.* (1976) reported a follow-up study of antibody titres in patients treated with HSV type 2 vaccine. Over a period of nine months the titres remained almost constant; this suggests an important role for cell-mediated immunity in herpes recurrences.

Jarisch and Sandor (1977) examined the migration inhibitory factor production of lymphocytes in patients with recurrent herpes simplex during therapy, and indicated that migration inhibition paralleled the therapeutic effect in the treated patients.

We studied the specific and non-specific lymphocyte transformation before vaccination and four months later. A stimulation index (SI) was obtained by dividing impulses per minute (ipm) by tritiated thymidine-uptake in lymphocytes of cultures with antigen by the ipm of cultures without antigen. The phytohaemagglutinin (PHA) stimulation index and the SI by herpes simplex antigen (HSV) remained constant (Wassilew, 1979).

One patient had no further recurrences after vaccination. Six out of the 10 patients had only mild and short-term recurrences, and the herpes-free interval was at least twice as long after vaccination at four weeks. In four patients the course of the disease remained constant.

Conclusion

We believe that vaccination is of value in the treatment of recurrent herpes genitalis. For a better knowledge of the mode of action of the vaccine the patient should be kept under continuous observation.

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Therapeutic aspects of herpes genitalis

A short review

J. SÖLTZ-SZÖTS AND D. FANTA

*From the Epidemiology Unit, Department of Dermatology II, University of Vienna, Vienna, Austria**

SUMMARY The treatment of manifestations of infection by herpes simplex hominis virus must be aimed at both the local eruptions and the mechanism of recurrence. Recently, some success has been achieved with the use of vaccines and other agents to stimulate a cell-mediated immunity in patients with recurrent herpes infection.

Local therapy

In recent years the administration of virucidal chemotherapy has increasingly gained ground; in this context it is important that administration starts in the prodromal or initial stage—that is, while the viruses are still multiplying.

ANTIMETABOLITES

Good results have been obtained with the antimetabolites, 5-iodo-2'-desoxyuridine, in ophthalmology. In dermatology, however, because of insufficient penetration of the agent good results can only be obtained in some cases after the addition of dimethylsulphoxide (DMSO).

TROMANTADINE HYDROCHLORIDE

At present there is no certain knowledge about the interaction between tromantadine hydrochloride, the virus, and the affected cell. Nevertheless, the administration of this compound at an early stage leads either to rapid drying of the herpetic manifestations in a high percentage of cases or to the eruptions subsiding in the prodromal stage (Fanta, 1976). The drawback to its topical administration is that it is a highly sensitising agent which frequently provokes contact dermatitis (Fanta and Mischer, 1976).

*In co-operation with the Ludwig Boltzmann Institute for Research on Infectious Venereodermatological Diseases.

Address for reprints: Professor J. Söltz-Szöts, Allgemeines Krankenhaus des Stadt Wien, II Universitäts-Hautklinik, 9 Alserstrasse 4, A-1090 Vienna, Austria

Received for publication 16 November 1978

FLUORESCENT DYES

The administration of heterocyclic fluorescent dyes to acute herpes simplex lesions followed by irradiation (photodynamic inactivation) was recommended for a while (Jarrat and Knox, 1975). There is, however, experimental evidence that photodamaged virus particles—that is, virus particles with DNA changes which no longer produce an infection—have the property of malignant transformation of cultured cells (Duff and Rapp, 1971).

Systemic therapy

IMMUNE STIMULATION

Attempts at influencing the mechanism of recurrence by immune stimulation are so far based on numerous, partly non-specific, partly specific vaccinations. It should be emphasised that the unpredictable course of the disease makes an evaluation of therapeutic measures difficult.

Herpes antigen

Considerable improvement can be achieved in 70%–80% of all cases by repeated administrations of herpes antigen. We have used a mixed tissue culture vaccine containing type 1 and type 2 antigens (Söltz-Szöts, 1971a) obtained from cultures of herpes type 1 and 2 in embryonated chicken eggs and subsequently heat-inactivated. The vaccine was standardised by determination of the virus titre with the complement fixation test (Söltz-Szöts, 1971b; Söltz-Szöts and Fanta, 1974).

Bacillus subtilis lysage

Bacillus subtilis lysage acts as an active stimulant of the reticuloendothelial system—that is, as a stimulant of immunospecific resistance—and consequently stimulates phagocytosis. Although it is recommended in the treatment of recurrent herpes there are no controlled studies on its efficacy.

Levamisole

Levamisole is a widely used anthelmintic agent; it is a chemical derivative of imidazol and has a stimulating effect on reduced cellular immunity (Symoens and Brugmans, 1974). In a double-blind study a significant improvement in recurrent herpes has been obtained (Fanta and Wimmer, 1979).

BCG vaccine

In the treatment of tumours as well as of chronic viral and bacterial infections, the stimulation of cell-mediated immunity by vaccination with BCG vaccine has become increasingly important. Furthermore, in the treatment of intractable cases of herpes genitalis a positive influence on the course of the disease could be achieved by repeated vaccinations with BCG vaccine. In an unselected group of patients with recurrent herpes a success rate of 60% has been observed; because of the possibility of strong local reactions, however, this treatment is justified only in cases that do not respond to other forms of therapy (Fanta and Wimmer, 1979).

Interferon

Interferon, with a broad antiviral spectrum, is species-specific, has a better prophylactic than therapeutic effect, and offers short-term protection. Clinical trials with exogenous interferon treatment of herpes virus infections in man have yielded promising results, but these still need to be confirmed on a larger scale by double-blind studies. More extensive clinical use of interferon would firstly require an expansion in production in order to ensure that all patients would receive treatment.

In order to achieve successful treatment with interferon-inducing agents the following criteria should be adopted:

- (1) Administration must start early; once the virus has stopped multiplying no further effect can be obtained.
- (2) The injections must be administered at 24-hourly intervals for a period of about eight days. The interferon-inducing agent must make direct contact with the cell and must, therefore, be administered in high dosages. In our studies of patients with extensive herpes simplex infections treated with an inactivated influenza virus as interferon-inducing agent, the average duration of the disease could be reduced. In addition, the intervals between herpetic recurrences were significantly prolonged when treatment was combined with herpes antigen vaccination (Söltz-Szöts, 1971a, b; 1976).

Conclusion

It must be emphasised, however, that despite great progress in the diagnosis, immunology, and treatment of herpes genitalis, we are still only at the beginning, promising as the progress may be.

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Theme 4: New trends in the epidemiology of STDs

Illness behaviour and sexually transmitted diseases

R. M. HARRISON

From the STD Research Project, Academic Department of Genitourinary Medicine, Middlesex Hospital Medical School, London

Introduction

No matter what talent or scientific skill, no matter what resources are devoted to the development of services for the treatment of sexually transmitted diseases (STDs), they may only operate effectively if those who can benefit present themselves for treatment. Endeavours to improve the quality of service in relation to sexually transmitted diseases, therefore, require that some attention be given to the question of illness behaviour.

For a long while it has been known that in general only a small proportion of people with treatable complaints actually bother to present themselves for treatment by a qualified medical practitioner. Even though this has been known, attempts to explain why some do and some do not go to the doctor have met with little success.

Attempts to find explanations, particularly in the more obvious and commonsensical directions such as the seriousness, the painfulness, or the inconvenience of the complaint, have failed; neither do the usual demographic variables such as age, sex, social class, nor ethnic background account for the major variations in illness behaviour. It is true that these variables do discriminate to some extent, but within any particular cultural or socioeconomic group variation exists, which, as yet, students of illness behaviour have largely been unable to explain.

There have, however, been some signs of hope in studies which have broken away from focusing on individual characteristics—be they psychological or socioeconomic—to focus on symptoms as perceived by potential patients in their everyday lives. That is, temporarily, the notions of sickness and health which belong primarily to professional medicine have been placed aside and attention has

been given to notions of what constitutes sickness and health, normal and abnormal, within the mind of the lay public. Alongside this has been the study of the ongoing relationship patients have with various members of the medical profession and, in particular, of their perceptions of the capabilities of medicine. Even though these lay notions might be regarded as mistaken or wrong by professional medical workers, they seem to offer the key, at least in the first instance, to understanding patients' behaviour.

Method

In order to test this framework, an investigative study of illness behaviour and STDs was carried out. For practical reasons, it was decided to work with a group who had delayed, rather than failed, to seek treatment. From an examination of records in a London clinic, it was noted that 75% of women consulted a doctor within six weeks of noticing a symptom, and men, within two weeks. A small group of men and women who had delayed seeking treatment beyond these time intervals were then interviewed. Interviews were unstructured and were tape-recorded with the consent of participants.

Results

The results of the pilot study showed that the approach outlined was of considerable value, and indicated the main areas on which further study should be focused; such as, the interpretation of bodily changes and the factors influencing these interpretations; alternative actions other than consulting a doctor; and the reasons for not pursuing an appropriate line of action.

Comment

The first task confronting individuals is to decide whether something is normal or abnormal. Changes

Address for reprints: Mr R. M. Harrison, Academic Department of Genitourinary Medicine, Middlesex Hospital Medical School, London W1

Received for publication 23 June 1978

in themselves are not always regarded as abnormal since a certain amount of variation is usual, particularly for women. Similarly, men may explain penile irritation as due to sexual vigour or excess.

Time is a very important criterion in diagnosis. If the lay diagnosis attributes the bodily changes to a specific situational event, such as excess sexual intercourse or wearing tight jeans, then making the appropriate adjustments to these situations should cure the problem. Time may defeat attempts to regard changes as normal. Changes can sometimes be accommodated on the basis of lay medical knowledge for an unlimited time. Hence, a man may explain away the growth on his penis because he tended to have growths elsewhere on his body.

Other people remain concerned about the context even when time defeats or challenges the initial diagnosis. Even though they may come to doubt their initial interpretation, if they do not regard themselves as appropriate candidates for certain types of diagnosis, then they may dismiss certain interpretations. To have a STD, they or their partners must have had a change of sexual contacts. If they do not believe this situation to exist then it may be difficult for them to contemplate STD as a diagnosis.

When someone finds their own lay medical knowledge exhausted, they do not always at that point rush to the doctor but instead may seek the advice of a friend. For example, they may consult a friend whom they regard as more knowledgeable than themselves. Similarly, girls may consult with their friends who have had similar problems.

It is known that people treat themselves. With STDs they may have pills left over from a previous attack, they may share pills, or they may resort to lay remedies, such as one girl who treated her

vaginal discharge by taking a bath with cider vinegar. We know that some ethnic minorities in Britain may consult a hakim or herbalist.

After going through these processes some people conclude that they may be suffering from something worthy of professional medical attention but do not seek such attention or delay in doing so. This may be for a variety of reasons. Obviously, some people have practical difficulties and if they do not regard themselves as in great medical danger will wait until it is convenient to seek care.

Fear and anxiety can be the basis for not seeking treatment sooner and may be categorised as follows:

- (1) Fear that they may have some serious illness, for example, cancer;
- (2) Fear of the treatment they might receive;
- (3) Fear of the stigma;
- (4) Fear of 'VD' as an illness itself.

Conclusion

Although a further large scale study is now under way to test the ideas outlined in a systematic and qualitative fashion from this small amount of work, tentative statements for health education may be suggested.

Health education in relation to sexually transmitted diseases will need to be finely balanced; that which may encourage some will discourage others. Certainly programmes which attempt to use fear to provoke people into action may be counter-productive.

Simple knowledge about the nature and effectiveness of treatment for STDs would be useful. Finally, there is a need to tackle the problem of stigma and sexually transmitted diseases.

Critical evaluation of the venereal disease control campaign in Poland 1970-76

J. TOWPIK

From the Institute of Venereology, Medical Academy, Warsaw, Poland

SUMMARY A comprehensive campaign to control venereal diseases was undertaken in Poland in 1970. During the eight-year period of the campaign a significant decrease in the morbidity rate of gonorrhoea and syphilis was achieved. The number of cases of primary and secondary syphilis decreased by 85% and the number of cases of gonorrhoea by 30%. This paper presents the main guidelines of the campaign and a critical evaluation of the various epidemiological methods applied, such as mass screening for syphilis and gonorrhoea, epidemiological treatment, contact-tracing, improvement of treatment methods, improvement of methods of health education, and training of physicians and laboratory staff in venereology.

Introduction

In Poland, as in many other European countries, a considerable increase in the morbidity rate for venereal diseases was observed in the years 1963-69. The Institute of Venereology in Warsaw, created in this connection in 1970, was charged with the task of applying all the means available to control the situation.

The general principles of the campaign undertaken at the time and its preliminary results have already been reported (Towpik, 1972; Towpik, 1976).

Now, after eight years, we can estimate the usefulness of the methods applied in this campaign.

Educational activities

Educational methods have changed considerably during the past eight years. After the period of intensive, wide-ranging propaganda from 1970 to 1972, undertaken to inform society as a whole on the dangers of venereal diseases and their social consequences, particular attention was paid in the following years to educational activities among young people and to collaboration with the Ministry of Education and with teachers.

Elementary education and guidance concerning venereology were introduced into many aspects of the school curriculum, including the study of biology, infectious diseases, and family life. A special handbook for teachers, tutors, and parents, which included many educational aspects related to venereal diseases, was produced. This handbook was a success and its fourth edition is in print. Furthermore, all pupils are presented with a free booklet before leaving elementary school (after eight grades).

In addition, special lectures with tape-recordings—sometimes with the participation of physicians—and films and slides approved by the Ministry of Education are given in schools. In some elementary schools, before the end of the eighth grade, public discussions and tests on general aspects of health, including venereological problems, are conducted. Such tests also provide information on the pupils' awareness of venereal diseases and their problems.

To stimulate journalists' interest in venereal diseases, a competition is held each year for the best educational and informative articles on venereal disease published in the daily press.

Films play an important part in our teaching. Up to 1977, 11 didactic colour films have been made; they are very helpful in teaching medical students and in broadening the knowledge of qualified doctors in the field of venereology. We have made eight educational colour films and 28 animated films, lasting two to three minutes, which are shown in cinemas throughout the country. Brief

Address for reprints: J. Towpik, Institute of Venereology, Medical Academy, ul. Koszykowa 82a, Warsaw 02-008, Poland

Received for publication 20 June 1978

talks are broadcast regularly on the radio every two weeks.

We are aware that in the field of educational activities we must constantly look for new, better, more attractive, and more effective forms in addition to the methods already in use.

Indeed, the effect of the educational work is very difficult to assess but a decrease in the morbidity of syphilis and gonorrhoea has occurred as well as a change in the attitude and behaviour of patients. They are consulting a doctor earlier than formerly and frequently they spontaneously bring their contacts for examination and treatment.

Contact-tracing

Contact tracing has always been difficult. A comparative study of this preventive work was made at the dermatovenereological centres in various areas to determine the extent of administrative shortcomings as well as the influence of different aspects of behaviour.

As a result of the inquiry it was established that the indices of contacts fluctuate as shown in Table 1. The index of contacts is the term used to describe the mean number of contacts for each patient.

The efficiency of contact-tracing was partially increased by improving interviewing techniques, and by collaboration with psychologists. The most important aspect, however, was the establishment of other factors, on which the Health Service has a very limited, or no, influence.

The main obstacles in the tracing of contacts were:

- (1) The promiscuity of anonymous contacts, many of whom were under the effect of alcohol;
- (2) The lack of co-operation of patients with the health service, because of low standards of awareness, lack of confidence in the function of the health service, and also fear of family or social complications;
- (3) The lack of conviction of the patients of the necessity of such co-operation, many of them giving false names and addresses.

In one agricultural province in order to increase the efficiency of contact-tracing, almost all the patients with symptomatic syphilis were admitted to hospital for a period of two to four days. A repeated history-taking under hospital conditions increased the index of named contacts by up to threefold. This method, however, cannot be generally applied.

The index of contacts of early syphilis for the whole country for the period 1969-77 is shown in Table 2 and that of gonorrhoea in Table 3.

Table 1 *Variation in index of contacts*

Diagnosis	Mean number of contacts per patient		
	Named	Examined	Diagnosed
Early syphilis	1.84-0.94	0.92-0.59	0.6-0.22
Gonorrhoea	0.85-0.52	0.88-0.53	0.66-0.5

Table 2 *Index of contacts of early syphilis*

Year	Mean number of contacts		
	Named	Examined	Diagnosed
1969	1.24	0.90	0.40
1970	1.42	0.90	0.41
1971	1.44	0.92	0.37
1972	1.12	0.89	0.41
1973	1.07	0.98	0.41
1974	1.07	0.95	0.46
1975	1.01	0.91	0.46
1976	1.03	0.90	0.43
1977	0.91	0.67	0.40

Table 3 *Index of contacts of gonorrhoea*

Year	Mean number of contacts per patient		
	Named	Examined	Diagnosed
1969	0.71	0.87	0.48
1970	0.75	0.88	0.41
1971	0.76	0.90	0.44
1972	0.71	0.88	0.48
1973	0.79	0.90	0.51
1974	0.74	0.89	0.54
1975	0.71	0.87	0.52
1976	0.69	0.88	0.50
1977	0.67	0.87	0.51

Periodical medical tests for venereal disease

Periodical medical tests for venereal infection followed by treatment when indicated were introduced for groups of individuals exposed to the risk of infection. Tests were applied to prostitutes, especially where they presented a potential epidemiological threat, such as, in sea ports, towns, and industrial centres. There were some objections to these examinations at first, but as time went on the number of individuals voluntarily reporting for examination at each clinic increased to several thousand a year. This response was achieved by a liberal and humane approach to the patients, who learned that such examinations did not entail punishment but instead offered them free and confidential medical care and the opportunity of receiving free treatment. As a result of these examinations the number of infectious cases in these groups decreased together with the risk of infection for their sexual partners.

For instance, in 1977, in the whole country there were 18 clinics of this kind, in which 70 000 tests were carried out leading to the diagnosis of 5000 cases of gonococcal infection and 150 cases of syphilis. Undoubtedly, these examinations reduced the morbidity rate for gonorrhoea and syphilis and the risk to sexual partners.

Epidemiological treatment

Each venereological centre was instructed to give epidemiological treatment to patients whose sexual contact with an infectious person was confirmed although no evidence of infection had been found. Follow-up examinations after one or two years of patients given epidemiological treatment for syphilis confirmed the efficacy of such management.

The index of contacts treated epidemiologically is presented in Table 4.

Mass serological screening

Preliminary statistical studies indicated that in 1969 of the total number of cases of syphilis about 30% were cases of recurrent secondary syphilis and about 22% were cases of early latent syphilis. This alarmingly high percentage of cases of syphilis not diagnosed in the early period of the disease stimulated an analysis of the reasons for this condition.

The studies conducted in several centres throughout the country indicated that the reasons for delayed consultations and late diagnosis of syphilis were:

- (1) A lack of instruction and knowledge of the early symptoms of the disease;
- (2) Fear or shame of acknowledging the disease;
- (3) Unsatisfactory self-treatment in the early period and the masking effect of drugs altering the course of the disease;
- (4) An atypical course of the disease from the onset in a number of patients, some of whom consulted general practitioners and received inappropriate local or general treatment which altered the course of the disease and delayed its diagnosis.

The results of these studies indicated the need to apply serological screening tests widely, with the purpose of tracing the cases of asymptomatic syphilis, and to institute intensive postgraduate training programmes in venereology for physicians.

With this aim mass serological screening of various social groups was undertaken. Apart from the routine examination of pregnant women, blood donors, and some professional groups, certain specific population groups were examined

Table 4 *Index of contacts treated epidemiologically*

Year	Mean no. of contacts per patient with	
	Early syphilis	Gonorrhoea
1972	0.29	0.19
1973	0.45	0.25
1974	0.67	0.31
1975	0.74	0.35
1976	0.62	0.36
1977	0.66	0.36

when it was justified epidemiologically. Routine serological examinations were also carried out for all patients in general hospitals between 15 and 65 years of age. The number of serological tests performed from 1969 to 1977 in this country is shown in Table 5. This type of examination proved to be very useful in finding cases of syphilis; most were cases of early and late latent syphilis and many patients were unaware of their disease.

The epidemiological and social importance of serological screening is illustrated by the fact that about 40% of all cases of syphilis were diagnosed by this method.

The difficulties connected with the control of gonorrhoea are well known among venereologists and do not need to be discussed here. Most of the epidemiological methods described above can be applied to this disease, but several amendments should be made. There should be:

- (1) Periodic evaluations of gonococcal susceptibility to the commonly prescribed antibiotics and adaptation of the treatment schedules to the results obtained;
- (2) Close co-operation between venereologists and gynaecologists and the participation of gynaecologists in mass screening tests for gonorrhoea.

The results from the United States as well as from Poland have shown that many cases of latent or complicated gonorrhoea in women may be detected in gynaecological clinics. This co-operation between clinics, as yet, is not satisfactorily established for psychological as well as technical reasons.

Table 5 *Number of serological examinations per year (1969-77)*

Year	No. of serological examinations
1969	3 740 000
1970	5 400 000
1971	6 390 000
1972	7 010 000
1973	7 650 000
1974	8 372 570
1975	8 149 472
1976	8 834 357
1977	8 859 885

In the past few years 80 new laboratories for the diagnosis of sexually transmitted diseases were added to the number of laboratories already working in this country. Nevertheless, this does not solve the problem and we need greater investment in laboratories and well-trained staff.

Results

Through these multidirectional activities the Institute of Venereology and all co-operative clinics and institutions have achieved considerable epidemiological success. In the eight years from 1970 to 1977 the incidence of primary and secondary syphilis declined by more than 85% (Fig. 1).

As far as gonorrhoea is concerned, our work is not yet complete. So far the spread of gonorrhoea

has been arrested and the incidence has decreased by 30% (Fig. 2).

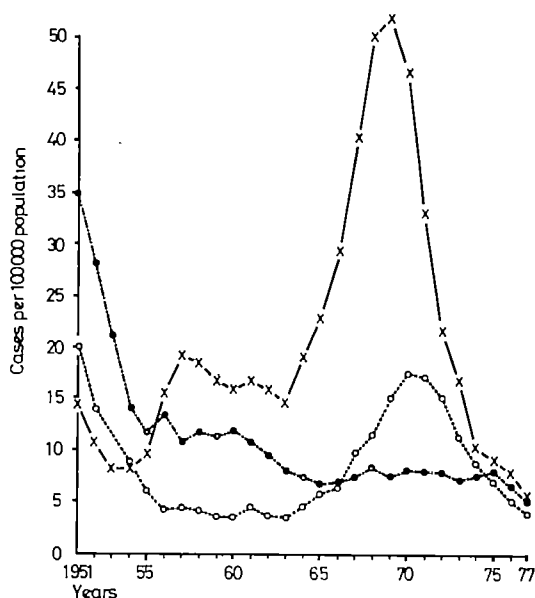


Fig. 1 Number of reported cases of syphilis from 1951 to 1977

×—× Primary and secondary syphilis; ○—○ early latent syphilis; ●—● late and late latent syphilis

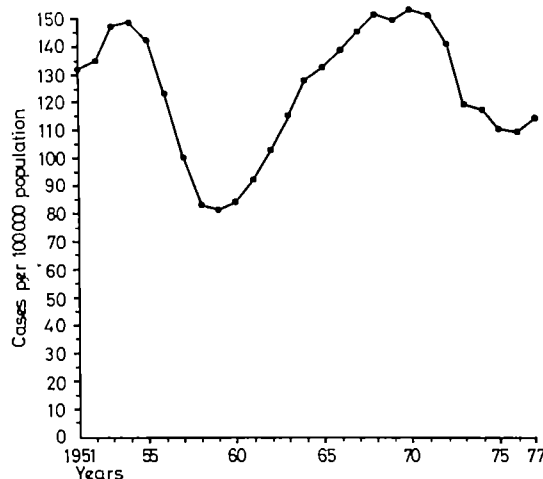


Fig. 2 Number of reported cases of gonorrhoea from 1951 to 1977

Conclusion

The epidemiological methods so far applied to control sexually transmitted diseases should be continued in the future, not as an element of a special campaign but as a permanent part of the health service. Nevertheless, their efficiency and usefulness should be periodically evaluated according to the epidemiological situation.

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Epidemiology of gonorrhoea and syphilis in South Australia (1966-77)

DON JORGENSEN

From the Venereal Disease Control Centre, Department of Public Health, Adelaide, South Australia

SUMMARY Data from the annual general report of the Venereal Disease Control Centre (VDCC) in a state with a population of approximately 1 250 000 people (in 1977), of whom about 1 100 000 live within 25 miles of the centre of Adelaide, the capital city, are presented.

Introduction

South Australia is the driest state in the driest continent in the world. Sparsely populated, the surface area is 380 000 square miles compared with that of the United Kingdom, which is 95 000 square miles. The aboriginal population living in the eight missions and other aboriginal centres are thought to number about 3000 (about 0.24% of the total), and they figure prominently in the epidemiology of syphilis because of the presence of endemic syphilis in these centres.

Incidence of syphilis

In 1966 legislation was passed requiring medical practitioners and laboratories dealing with syphilis or gonorrhoea to notify numbers of cases to the Central Board of Health. The incidence of reported syphilis has risen from 1.4/100 000 in 1966 to 40/100 000 in 1976 (Table 1); this figure dropped to 28/100 000 in 1977. The rise in 1976 was almost entirely due to increased efficiency in collecting blood specimens from aboriginals in that year.

The overall incidence for Australia has dropped behind the South Australian figures since the early 1970s, not because South Australia has more cases of syphilis but because the figures are more accurate.

Incidence of gonorrhoea

Since 1966 the incidence of gonorrhoea has risen from 23/100 000 to 174/100 000 in 1974 and is now around 150/100 000; the steep rise in incidence

since 1966 seems to have levelled out over the last four years (Table 2). There are, no doubt, two reasons for this increase: an actual increase in the number of cases and an increase in efficiency in collecting statistics. The overall incidence of 85/100 000 in Australia in 1976 and 1977 shows

Table 1 *Notification of cases of syphilis from 1966 to 1977. (Populations in 1977 of Australia and South Australia were 14½ million and 1½ million respectively)*

Year	South Australia	Rate per 1000 000	Australia	Rate per 100 000
1966	15	1.4	1080	9.3
1967	21	1.9	845	8.0
1968	58	5.2	860	7.1
1969	38	3.3	1072	8.6
1970	74	6.4	952	7.9
1971	122	10.4	1047	8.3
1972	205	17.0	1215	9.3
1973	178	15.0	1433	10.9
1974	257	21.5	1864	13.9
1975	309	25.0	2362	18.0
1976	501	40.0	3110	23.0
1977	360	28.1	3023	21.5

Table 2 *Notification of cases of gonorrhoea from 1966 to 1977*

Year	South Australia	Rate per 100 000	Australia	Rate per 100 000
1966	355	23	8553	74
1967	599	51	9412	80
1968	509	43	9763	79
1969	703	61	9648	79
1970	650	55	9558	76
1971	817	70	10274	81
1972	989	83	11017	85
1973	1492	126	11357	85
1974	2091	174	12524	95
1975	2121	172	12511	93
1976	1885	151	11526	85
1977	1921	150	12024	85

Address for reprints: D. Jorgensen, VD Control Centre, Department of Public Health, 275 North Terrace, Adelaide, S. Australia 5000

Received for publication 2 September 1978

that our national figures do not reflect the prevalence of gonorrhoea in the country as a whole (Table 2).

It is not possible to estimate how many cases of urethral discharge are treated empirically, and these figures do not, therefore, enter the statistics; but there are, however, many cases in South Australia and, obviously, more in Australia as a whole.

Epidemiological data of notified cases

AGE GROUPS

For cases of gonorrhoea the predominant age group is 15-19 years in women and 20-24 years in

men. These two groups were responsible for 68% of cases of gonorrhoea in 1977 (Table 3).

The age range for syphilis, on the other hand, is greater, but those figures include both active and inactive notified cases; most of the active syphilis occurs in the younger age groups (Table 3).

MARITAL STATE

In 1977, 1577 (80%) out of 1921 cases of gonorrhoea occurred in single people. The figures for syphilis were more evenly divided but include both active and inactive cases (Table 4).

SOURCES OF INFECTION

Friends or fiancés or casual contacts account for

Table 3 *Notifications and incidence of gonorrhoea and syphilis by age and sex (1977)*

Age (years)	Gonorrhoea			Syphilis		
	Male	Female	Total	Male	Female	Total
0-9	2	1	3			
10-14	1	15	16		2	2
15-19	312	304	616	15	30	45
20-24	454	238	692	56	76	92
25-29	239	75	312	60	76	86
30-39	169	48	217	54	16	70
40-49	38	7	45	19	6	25
50 and over	15	5	20	27	13	40
Total (1977)	1228	693	1921	231	129	360
Total (1976)	1145	740	1885	299	202	510
Total (1975)	1296	825	2121	164	140	304

Table 4 *Marital state of notified cases of gonorrhoea and syphilis*

Marital state	Gonorrhoea									Syphilis								
	Male			Female			Total			Male			Female			Total		
	1975	1976	1977	1975	1976	1977	1975	1976	1977	1975	1976	1977	1975	1976	1977	1975	1976	1977
Single	1051	886	1045	657	546	532	1708	1432	1577	87	122	141	54	76	72	141	198	213
Married	158	165	122	92	93	85	250	258	207	33	120	79	47	103	53	80	223	132
Defacto	19	22	9	16	26	12	35	48	21		4		1	3		1	7	
Widowed	3	1	1	7	2		10	3	1					1	1		1	1
Divorced	16	8	3	10	18	19	26	26	22		1	1		1			2	1
Separated	42	58	45	42	53	45	84	111	90	3	2	2	1	2	2	4	4	4
Unknown	7	5	3	1	2		8	7	3	41	50	8	37	16	1	78	66	9
Total	1296	1145	1228	825	740	693	2121	1885	1921	164	299	231	140	202	129	304	501	360

Table 5 *Source of infection of notified cases of gonorrhoea and syphilis*

Contact	Gonorrhoea									Syphilis								
	Male			Female			Total			Male			Female			Total		
	1975	1976	1977	1975	1976	1977	1975	1976	1977	1975	1976	1977	1975	1976	1977	1975	1976	1977
Marital partner	32	41	17	70	54	40	102	95	57	3	3	1	9	5	5	12	8	6
Defacto partner	15	19	9	17	20	7	32	39	16	1	2		1	2		2	4	
Friend/fiancé	356	294	341	319	289	326	675	583	667	3	11	14	5	10	5	8	21	19
Casual contact	741	589	560	394	317	257	1135	906	817	45	42	30	19	16	7	64	58	37
Prostitute	24	39	38				24	39	38	1	4					1	4	
Homosexual	58	60	145				38	60	145	6	20	17				6	20	17
Mother	1	4	2		2		1	3	2		1						1	
Unknown		102	116		58	63		160	179	116	216	169	100	169	112	216	385	281
Total	1227	1145	1228	800	740	693	2027	1885	1921	175	299	231	134	202	129	309	501	360

77% of sources of infection for gonorrhoea and 21% for syphilis; unknown sources of the latter account for 78% of cases (Table 5). This high unknown factor is due to the difficulties of contact-tracing in the aboriginal population. A feature of both gonorrhoea and syphilis is the substantial number of homosexuals affected—8% overall for gonorrhoea (12% in males) and 5% overall for syphilis (8% in males).

USUAL RESIDENCE

Eighty-eight per cent of patients with gonorrhoea lived within 25 miles of the centre of Adelaide. Patients with syphilis were more or less evenly distributed, because of the aboriginal missions (Table 6).

COUNTRY OF ORIGIN

Table 7 shows only the numbers of cases from each country of origin. The percentages of the total population for the groups is not known. The aboriginal population accounted for 41% of cases of reported syphilis in 1977.

OCCUPATION

The highest number of notifications came from the less skilled and unemployed members of the community (Table 8); the actual percentages in the groups are not known. Among the cases of gonorrhoea 2.5% gave their occupation as prostitution.

Notification of cases of syphilis by stage of disease

In Table 9 notifications of cases of syphilis have been subdivided into stages of the disease in aboriginal missions (in brackets) and in the general population. These are difficult figures to extract but they do show trends. For the aboriginals it is not possible to give figures for primary and secondary syphilis because of the lack of clinical details.

Diagnosis is made by consultation between the serologist and the venereologist at the central laboratory, at the Institute of Medical and Veterinary Science, on the results of the serological tests. The nursing sisters, who live on the missions, are then given instructions by the venereologist on the treatment required. In the general population the prevalence of primary or early active syphilis is greater in men than in women and this agrees with experience in most countries.

Aboriginals living on reserves (0.24% of the total population) were responsible for 149 (41%) of 360 cases of syphilis notified in 1977.

Sources of notified cases

The VDCC notified 1142 (60%) of 1921 cases of gonorrhoea and 186 (52%) of 360 cases of syphilis, including cases from the out-back and aboriginals (Table 10). Until recently South Australia had the

Table 6 Usual residence of notified cases of gonorrhoea and syphilis

Residence	Gonorrhoea									Syphilis								
	Male			Female			Total			Male			Female			Total		
	1975	1976	1977	1975	1976	1977	1975	1976	1977	1975	1976	1977	1975	1976	1977	1975	1976	1977
Metropolitan	1122	968	1072	783	693	632	1905	1661	1704	80	132	142	55	70	52	135	204	194
Country	154	162	144	39	43	58	193	205	202	78	161	87	85	131	76	163	292	163
Interstate	9	12	10	3	4	3	12	16	13	1	1	1		1	1	1	2	2
Overseas	11	3	2				11	3	2	5	3	1				5	3	1
Total	1296	1145	1228	825	740	693	2121	1885	1921	164	299	281	140	202	129	304	501	360

Table 7 Country of origin of notified cases of gonorrhoea and syphilis

Country of origin	Gonorrhoea									Syphilis								
	Male			Female			Total			Male			Female			Total		
	1975	1976	1977	1975	1976	1977	1975	1976	1977	1975	1976	1977	1975	1976	1977	1975	1976	1977
Australia	888	761	915	683	622	605	1571	1383	1520	73	121	109	49	58	46	122	179	155
Aboriginals	108	106	54	35	36	20	143	142	74	64	148	72	77	132	77	141	280	149
United Kingdom	94	77	87	57	38	32	151	115	119	3	4	5		1	1	3	5	6
Mediterranean																		
Europe	115	123	96	26	21	19	141	144	115	12	6	13	6	3	2	18	9	15
Other Europe	65	57	55	15	22	15	80	79	70	10	11	5	5	8	2	15	19	7
Asia	23	10	15	5	1	2	28	11	17	2	6	27			1	2	6	28
America																		
Other	3	11	6	4			7	11	6		3		3			3	3	
Total	1296	1145	1228	825	740	693	2121	1885	1921	164	299	231	140	202	129	304	501	360

enviable position whereby syphilis serum antibody tests, other than reagin screening tests, were done in one laboratory, at the Institute of Medical and Veterinary Science, which resulted in a very accurate notification system together with standard methods and efficient control in serological tests. Accurate and consistent reporting of serological test results and accurate record-keeping of the serological history of syphilitic patients were possible. Now that syphilis serological tests are carried out in various other laboratories this position has altered, the main reasons being the difficulties in keeping

the syphilis registry up to date and the differing laboratory standards.

TYPES OF CONTACTS

In addition to friends or fiancés or casual contacts, a high number of sexual contacts are homosexual, 244 (15%) of 1606 cases with gonorrhoea and 29 (31%) of 94 cases of syphilis (Table 11).

CONTACT-TRACING

In 1977, of 2570 contacts of gonorrhoea 842 (32%) were not sought, mainly because of insufficient

Table 8 *Occupation of notified cases of gonorrhoea and syphilis*

Occupation	Gonorrhoea						Syphilis					
	Male		Female		Total		Male		Female		Total	
	1976	1977	1976	1977	1976	1977	1976	1977	1976	1977	1976	1977
Rural		41		3		44		11				11
Artist/entertainer		37		6		43		1				1
Professional	14	16	4		18	16		2				2
Technical		15		3		18						
Managerial	50	45	3	5	53	50	7	3			7	3
Skilled	168	176	13	14	181	190	6	9			6	9
Apprentice	13	31		2	13	33	1				1	
Semi-skilled	140	84	23	21	163	105	3	6	2		5	6
Unskilled	242	211	13	18	255	229	1	45	1	7	2	52
Clerical	121	143	188	134	309	277	21	18	11	6	32	24
Sales	78	98	49	67	127	165	7	17	4	1	11	18
Maritime												
Student { Primary												
{ Secondary	55	55	80	82	135	137	4	5	2	2	6	7
{ Tertiary												
Nursing	7	11	47	38	54	49	1		1		2	
Armed forces	8	14		1	8	15		5	1		1	5
Home duties			120	115	120	115			52	39	52	39
Prostitution			48	47	48	47			2	4	2	4
Retired	4	2	19	21	23	23		27	14	14	14	41
Unemployed/unknown	121	108	81	72	202	180	245	76	111	56	356	132
Teaching	6	20	7	7	13	27	3	3			3	3
Transport	68	72	4	6	72	78		3				3
Service worker	50	49	41	31	91	80			1		1	
Total	1145	1228	740	693	1885	1921	299	231	202	129	501	360

Table 9 *Number of cases of syphilis by stage of disease (figures for aboriginals in brackets)*

Syphilis	Male			Female			Total		
	1975	1976	1977	1975	1976	1977	1975	1976	1977
Primary	45 (7)	56 (7)	20 (7)	13 (7)	18 (7)	3 (7)	58 (7)	74 (7)	23 (7)
Secondary	8 (7)	6 (7)	3 (7)	4 (7)	4 (7)	1 (7)	12 (7)	10 (7)	
Early active (other)	61 (53)	111 (94)	90 (37)	70 (45)	98 (77)	61 (41)	131 (98)	209 (171)	151 (78)
Total early	114 (53)	173 (94)	113 (39)	87 (45)	120 (77)	65 (41)	201 (98)	293 (171)	178 (80)
Late	2	27	2	4	14	2 (1)	6	41	
Old inactive	19 (4)	80 (19)	92 (22)	15 (6)	57 (16)	53 (30)	34 (10)	137 (35)	145 (52)
Not known	29 (3)	19 (8)	24 (11)	34 (2)	11 (3)	9 (5)	63 (5)	30 (11)	33 (16)
Total late	50 (7)	126 (27)	118 (33)	53 (8)	82 (19)	64 (36)	103 (15)	208 (46)	182 (69)
All syphilis	164 (60)	299 (121)	231 (72)	140 (53)	202 (96)	129 (77)	304 (103)	50 (217)	360 (149)

information. Of 1728 contacts sought, 1431 (82%) were found; of 1431 found, 929 (65%) were infected (Table 12). In 1977, of 120 contacts of syphilis 54 (45%) were not sought; of 66 contacts sought, 59 (90%) were found; and, of 59 contacts found, 26 (44%) were infected.

The ratio of male-to-female cases of gonorrhoea was 2:1 compared with 1.5:1 in 1976. This ratio indicates the efficiency of contact-tracing methods, the ratio falling as efficiency increases.

NOTIFICATION BY PRIVATE DOCTORS

Tables 13a and 13b show a static position as regards notifications of cases of gonorrhoea and syphilis by private practitioners, which may not change much under present conditions. Two areas of effort which may improve notifications are: (1) increased efforts to show undergraduates and graduates the reasons for, and importance of, early notification; and (2) an easier method of notification, namely, a simple notification form attached to an addressed, pre-paid envelope marked private and confidential to be used by all private practitioners and laboratories.

ATTENDANCES AT THE VENEREAL DISEASE CONTROL CENTRE

An increase from 12 674 to 14 204 (12% increase) patients occurred from 1976 to 1977 (Table 14); this is an increase in the daily average from 50 to 56 cases (12% increase), and means that from 30 to 90 patients were seen each day. With the present staffing levels medical officers have difficulty in maintaining adequate standards.

GONORRHOEA

Of 1738 notifications from the metropolitan area 1102 (63%) were seen at the VDCC (Table 15).

NON-SPECIFIC URETHRITIS

Non-specific urethritis is not readily distinguishable from gonorrhoea and other causes of urethritis in men without laboratory investigation, and although not a notifiable disease it is included in statistics from the UK and other countries. The total number of cases of non-specific urethritis seen at the VDCC in 1976 and 1977 are compared with that of male cases of gonorrhoea in Table 15.

Table 10 Sources of notifications for gonorrhoea and syphilis

	Gonorrhoea									Syphilis								
	Male			Female			Total			Male			Female			Total		
	1975	1976	1977	1975	1976	1977	1975	1976	1977	1975	1976	1977	1975	1976	1977	1975	1976	1977
Private doctors	475	414	407	214	228	197	689	642	604	38	93	87	37	59	45	75	152	132
RAH and VDCC clinics	691	694	709	520	434	433	1211	1038	1142	32	44	76	21	18	11	53	62	87
RAH other	2	6	4	18	22	14	20	28	18	6	11	3	7	7	7	13	18	10
QEH	20	16	17	36	18	28	56	34	45	7	5	4	4	9	8	11	14	12
QVH				9	8	6	9	8	6				2	1	2	2	1	2
Port Adelaide clinic	93	90	80	11	24	10	104	114	90	10	6	6		2		10	8	6
Armed forces	1		1				1		1									
Prisons	1		1	1														
Outback RFDS, AIM etc.	1	2					1	2		58	129	47	59	103	52	117	232	99
Other	12	13	9	16	6	5	28	19	14	13	11	8	10	3	4	23	14	12
Total	1296	1145	1228	825	740	693	2121	1885	1921	164	202	129	140	202	129	304	501	360

Table 11 Types of contacts noted (other than aboriginals in reserves)

Contacts	Gonorrhoea									Syphilis								
	Male			Female			Total			Male			Female			Total		
	1975	1976	1977	1975	1976	1977	1975	1976	1977	1975	1976	1977	1975	1976	1977	1975	1976	1977
Casual	850	676	662	620	549	397	1470	1216	1059	52	45	38	23	32	12	75	77	50
Marital partners	107	114	77	85	75	60	192	189	137	9	6	3	9	6	5	18	12	8
Defacto partners	24	27	10	23	31	12	47	58	22	1	4	1	1	3		2	7	1
Friend/fiancé	630	501	571	486	506	531	1116	1007	1102	10	26	23	6	19	9	16	45	32
Homosexual	97	109	244				97	109	244	11	62	29				11	62	29
Prostitute	26	41	40				26	41	40	1	4					1	4	
Mother	1	1	2		2		1	3	2		1						1	
Total	1735	1469	1606	214	1154	1000	2949	2623	2606	84	148	94	39	60	26	123	208	120

Table 12 *Contact-tracing*

<i>No. of contacts</i>	<i>Gonorrhoea</i>								<i>Syphilis</i>							
	<i>Contacts of male cases</i>				<i>Contacts of Female cases</i>				<i>Contacts of male cases</i>				<i>Contacts of Female cases</i>			
	<i>Female</i>		<i>Male</i>		<i>Male</i>		<i>Total</i>		<i>Female</i>		<i>Male</i>		<i>Male</i>		<i>Total</i>	
	1976	1977	1976	1977	1976	1977	1976	1977	1976	1977	1976	1977	1976	1977	1976	1977
Noted	1360	1362	109	244	1154	1000	2623	2570	86	65	62	29	60	26	208	120
Not sought	458	449	45	70	504	323	1007	842	27	33	32	10	14	11	73	54
Sought not found	207	202	15	53	83	78	305	333	18	5	8		8	2	34	7
Found	695	711	49	121	567	599	1311	1431	41	27	22	19	38	13	101	59
Diagnosed as infected	404	416	28	87	423	426	855	929	22	11	5	9	26	6	53	26
Treated epidemiologically	270	141	20	19	107	104	397	264	13	6	5	4	5	2	23	12

Table 13a *Number of private doctors notifying cases of gonorrhoea or syphilis or both (numbers given in brackets refer to doctors notifying cases for the first time)*

<i>Year</i>	<i>Area of practice</i>			<i>No. of Doctors previously involved*</i>	<i>PMS Doctors†</i>
	<i>Metropolitan</i>	<i>Country</i>	<i>Total</i>		
1966	10	7	17		560
1967	51	28	79	NA	580
1968	121	38	159	NA	594
1969	140	39	179	131	615
1970	134	38	172	191	623
1971	185 (52)	57 (31)	215 (83)	215	638
1972	164 (79)	69 (35)	233 (114)	311	658
1973	225 (141)	60 (17)	285 (158)	245	690
1974	293 (96)	72 (22)	365 (118)	221	700
1975	270 (85)	73 (29)	343 (114)	241	700
1976	284 (90)	69 (21)	353 (111)	230	700
1977	279 (84)	71 (19)	350 (103)	235	

*Doctors who have notified cases previously but not in the particular year

†Doctors on Pensioner Medical Service Register at June 30 1977

NA not applicable

Table 13b *Number of cases of gonorrhoea and syphilis notified by general practitioners*

<i>Year</i>	<i>Gonorrhoea</i>		<i>Syphilis</i>	
	<i>Males</i>	<i>Females</i>	<i>Males</i>	<i>Females</i>
1975	475	214	38	37
1976	414	228	93	59
1977	407	197	87	45

Table 14 *Number of cases seen at the Venereal Diseases Control Centre in 1977 (figures for 1976 are given in brackets)*

	<i>Jan.</i>	<i>Feb.</i>	<i>Mar.</i>	<i>Apr.</i>	<i>May</i>	<i>Jun.</i>	<i>Jul.</i>	<i>Aug.</i>	<i>Sep.</i>	<i>Oct.</i>	<i>Nov.</i>	<i>Dec.</i>	<i>Total</i>
Male patients													
Total	678	858	748	610	718	656	598	601	869	769	838	662	8605 (7507)
Average/day	35	43	33	34	34	31	28	26	40	38	38	33	34.4 (29.8)
New patients/episodes	378	426	366	311	365	327	289	354	451	380	418	349	4414 (3884)
Average/day	20	21	16	17	17	16	14	15	21	19	19	18	17.7 (15.5)
Female patients													
Total	405	503	512	426	495	495	408	518	522	463	523	329	5599 (5167)
Average/day	21	25	22	24	24	24	19	23	24	23	24	17	22.5 (20.4)
New patients/episodes	187	225	186	163	192	163	168	206	234	204	202	137	2267 (2168)
Average/day	10	11	8	9	9	8	8	9	11	10	9	7	9.0 (8.5)
Total no. of patients													
Total	1083	1361	1260	1036	1213	1151	1006	1119	1391	1232	1361	991	14 204 (12 674)
Average/day	56	64	55	58	58	55	47	49	64	61	62	50	56.5 (50.2)
New patients/episodes	565	651	552	474	557	490	457	560	685	584	620	486	6681 (6052)
Average/day	30	32	24	26	26	24	22	24	32	29	28	25	26.8 (23.2)

Table 15 Number of notified cases of gonorrhoea and non-specific urethritis from different sources

	<i>Country</i>		<i>VDCC</i>		<i>Metropolitan</i>		<i>Total (1977)</i>
	1976	1977	1976	1977	1976	1977	
Gonorrhoea							
Male cases		133	589	681		1095	1228
Female cases		50		421		643	693
Total		183		1102		1738	1921
Non-specific urethritis			1206	1375			

Epidemiology of gonorrhoea and syphilis in New Zealand

W. M. PLATTS

From the Department of Health, Christchurch, New Zealand

Introduction

New Zealand is a narrow, isolated country of two main islands covering 1000 miles of latitude. Over half of its 2 100 000 population live in the northern half of the north island near Auckland, its biggest city of 750 000 people.

Of the population 9.8% are Maoris and 2% are Polynesians fairly recently arrived from the Pacific Islands; again, most are concentrated in the warmer north.

Integration of the venereal disease services on the British pattern began in 1964, and there are now 13 clinics run as outpatient services by hospital boards. More clinics, however, are needed. All are staffed by part-time venereologists, seven of whom have taken the Liverpool or London Diploma using a government award scheme. There is a contact-tracing service using health department nurses, two of whom are based at their respective clinics.

A yearly meeting of medical public health and laboratory personnel concerned with venereology and sponsored by the health department has been held since 1964; furthermore, a three-week polytechnic training course for venereal disease clinic workers has just been started. Undergraduate teaching is poor, although most medical students are now rostered to attend clinics for practical instruction. Didactic instruction is minimal.

A 'dial-a-VD' service now operates in 10 cities and is being extended, but in general much more could be done in the sphere of public education.

As in the UK, venereal disease is not notifiable, but infectious syphilis is being voluntarily notified by private laboratories. Sexual contacts in New Zealand can be compulsorily examined, a law which is seldom put into practice. Methods have been standardised with full investigations and follow up, according to the standards recommended in British clinics. Laboratory standards in New

Zealand are high, with quality control being exercised from a reference laboratory.

Gonorrhoea treatment schedules are based on a single injection of 2.2 megaunits of fortified procaine penicillin with probenecid, which has resulted in very few failures at present. Antibiotics are obtained by prescription only, and the use of spectinomycin is confined to clinics and only used for β -lactamase-producing isolates and some patients who are hypersensitive to penicillin. Up to March 1978 16 isolates (12 proved) producing β -lactamase had been reported; these were mostly from patients from the Far East, but no sexual contacts were found. This figure compares with a total of 37 cases found in Australia up to October 1977, 14 being from the Far East (personal communication).

Syphilis

INCIDENCE

The incidence of infectious syphilis is at present low in New Zealand (2.8 per 100 000 population, a total of under 100 cases a year). It is mostly introduced by visitors from overseas, but local outbreaks among urban homosexual groups have occurred; since both of these groups are growing an increase in cases of infectious syphilis is likely in the future.

Most adults from the Pacific islands have had yaws and were treated in 1958 and 1962 by the World Health Organisation. Infectious syphilis has recently appeared among them, however, and indicates a disappearance of their immunity.

Gonorrhoea

INCIDENCE

All diagnosed cases of gonorrhoea (clinic cases together with those referred by general practitioners via private laboratories) in New Zealand totalled 6482 in 1976, a rate of 208 per 100 000 population; however, the cases seen by general practitioners and not diagnosed could swell this total by a further 25% at least.

Address for reprints: Dr W. M. Platts, Department of Venereology, Christchurch Hospital, Christchurch, New Zealand

Received for publication 1 August 1978

Of the total number of diagnosed cases throughout the country 50% are now seen in clinics, a figure which would be much higher if the clinic facilities in our biggest city, Auckland, were brought into line with the other three cities.

As has happened in many European countries, the upward trend in the incidence of gonorrhoea has fallen over the past two years (Fig. 1), the male-female ratio having remained steady at about 1.75:1 (Fig. 2). Recidivists are not a great problem in New Zealand clinics and attendances for surveillance are reasonable.

Gonococcal ophthalmia is uncommon, but pelvic inflammatory disease (PID) is common, comprising 19% of all female cases seen at the Christchurch clinic in 1977. Most of these cases were gonococcal.

Disseminated gonococcal infection is rare. Most cases, therefore, are being missed and presumably cured by routine antibiotic treatment in medical wards, which indicates the sensitivity of the gonococcus in this condition.

SCREENING AND SURVEYS

Routine screening for gonorrhoea is practised in some public obstetric and gynaecological clinics, in women's prisons, in all child welfare girls' homes, and, experimentally, in one family planning association clinic. The yield of positive results in the latter clinic is 0.5% from 2402 women seen over six months; one half of these were married, the other half single.

A large survey at the Christchurch Women's Hospital obstetric and gynaecological clinics, in

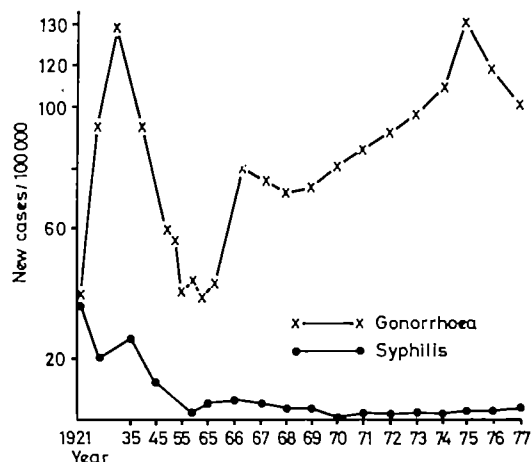


Fig. 1 Incidence of gonorrhoea and syphilis from 1921 to 1977 in New Zealand clinics (from 1971 rate for infectious syphilis only is shown)

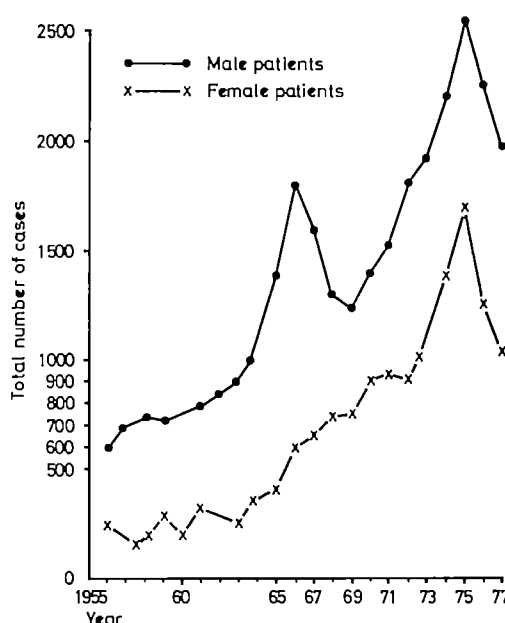


Fig. 2 Total number of male and female cases of gonorrhoea from 1955 to 1977 in New Zealand clinics

which 3389 cases were examined between 1970 and 1973 (Platts and Cresswell, 1974), yielded a positivity rate of 2.4% among the so-called 'single' group (that is, both unmarried or separated women) and a rate of 0.7% among the married group. Since then surveys have shown lower rates, but the decline may be due to technical problems.

Gonococcal sensitivities have been monitored since 1967. Initially relatively high values were obtained owing mainly to technical problems. From 1971 to 1975 the minimum inhibitory concentrations (MICs) were low but in 1976 and 1977 there was an unexplained slight rise. In 1977 26.6% of 2271 isolates had an MIC >0.1 IU/ml (Fig. 3).

Other sexually transmitted diseases (STDs)

NON-SPECIFIC URETHRITIS

The ratio of cases of non-specific urethritis (NSU) to male cases of gonorrhoea is 1.7:1, the upward trend levelling out for the first time in 1977 (Fig. 4). Recurrences due to relapse or reinfection are high (40% in one series over a year). NSU is the commonest STD infection found among university students. The large number of young men, many of whom return again and again and require time-consuming counselling to avert an anxiety state, comprise a serious STD problem in New Zealand today.

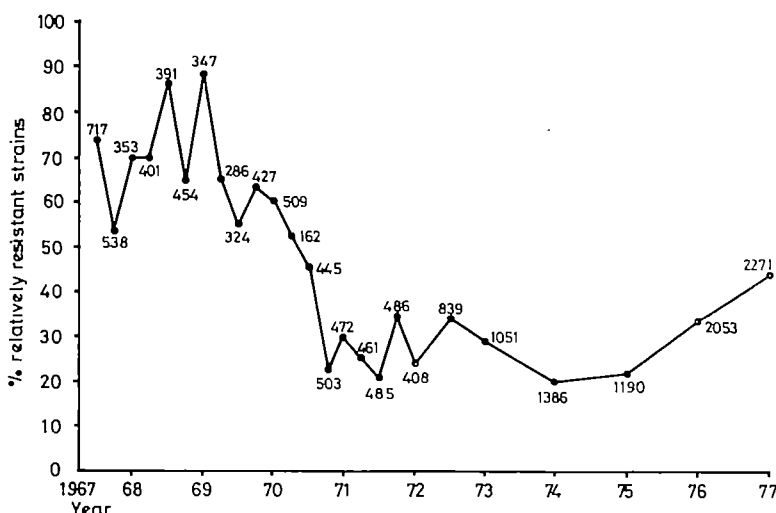


Fig. 3 Penicillin resistance of gonococci in New Zealand. (Relatively resistant strains mean strains with an MIC of >0.1 units of penicillin per ml.) (The numbers give the total numbers of strains tested each year.)

Most sexual contacts of NSU are examined and treated epidemiologically, since there is no laboratory service for the culture of *Chlamydia* in the country yet.

GENITAL HERPES AND GENITAL WARTS

The two viral diseases, genital herpes and genital warts, have become increasingly common over the last few years and show no sign of waning. They are time-consuming and demanding to treat. A viral culture service is available but there are often delays in obtaining results.

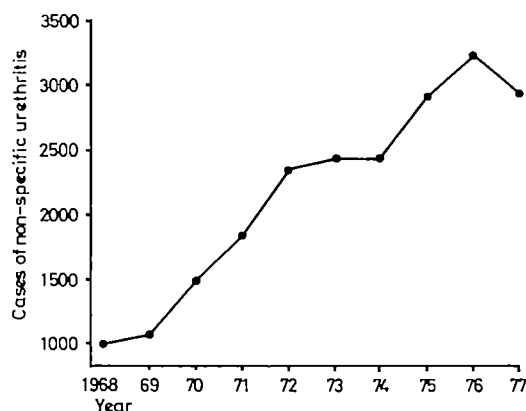


Fig. 4 Total number of male cases of non-specific urethritis from 1968 to 1977 in New Zealand clinics

TRICHOMONIASIS AND CANDIDOSIS

Trichomoniasis is relatively uncommon in New Zealand (4% in a large survey of obstetric and gynaecological clinic patients) whereas the incidence of candidosis (at 21% in the same series) is comparable to overseas figures.

HEPATITIS B

Sexually transmitted hepatitis B has been found in patients attending clinics (Stringer *et al.*, 1978) but cytomegalovirus has not yet been sought.

The minor types of STD are now being routinely reported from all clinics in New Zealand, the rates comparing closely with those given for England in 1975 (Department of Health and Social Security, 1978) (Table 1).

Table 1 Incidence of 'minor' sexually transmitted diseases in New Zealand clinics in 1977 compared with that in England in 1975

Diagnosis	Rate per 100 000 population	
	New Zealand (1977)	England (1975)
Non-specific urethritis (male patients)	300	306
Candidosis (female patients)	79.6	75.5
Trichomoniasis (female patients)	37.5	77.03
Genital warts	27.3	43.38
Herpes genitalis	20.0	13.38
Pubic lice	14.7	11.01
Scabies	6.6	6.02
Molluscum contagiosum	3.5	1.62

Epidemiology

'AT RISK' GROUPS

Age

Between 50% and 60% of female and 25%–30% of male patients attending clinics are teenagers. The number of schoolgirls has increased from 3% to 17% over the last six years at the Christchurch clinic (Table 2).

In a recent survey of 1300 clinic attenders and controls from a polytechnic college, three-quarters of the male patients attending the clinic and over half of the female patients stated that they had first had sexual intercourse aged 16 or younger as did half the male controls. The survey also showed that clinic attenders, especially the girls, left school at a younger age with a lower grading (nearly 50% at 16 or younger at fourth form level or lower) and that they were more likely to come from broken homes.

Table 2 Incidence of gonorrhoea in New Zealand clinics in 1977 by age group

Age group (years)	Rate per 100 000 population	
	Male	Female
10–14	4.8	21
15–19	471	557
20–24	744	339
25–29	535	225
30–39	160	0

Occupation

The bands of seasonal workers that move about the country—mixed shearing gangs of over 100, fruit pickers, hydroelectric engineering project workers, also female tourist hotel workers—many of whom are often itinerant and come from Australia, are high risk groups and because of often being out of reach of clinics pose a control problem. Groups of female 'shippies', mostly Maori, accompany over-

seas ships from one port to another; they have a high infection rate and are hard to trace.

Prostitution, which has never before been a social problem in New Zealand—(reputedly) because of the numbers of enthusiastic amateurs—has started on a large scale in the four main centres under the guise of massage parlours with telephone call-out services. Already, many of these girls are becoming infected.

Ethnic groups

The venereal disease rate among Maoris and Polynesians is two to four times that among whites.

Homosexuals

As increasing numbers of male patients attending clinics are found to be homosexuals, the extent of their involvement in the transmission of sexually transmitted diseases is becoming clearer.

Conclusion

A venereal disease service on the British model has been operating over the past 14 years in New Zealand. Despite the fee-for-service method of payment of the general practitioner—which places him in direct competition with the clinic for patients—the system has accomplished a great deal and clinics now see two-thirds of the total number of diagnosed cases of gonorrhoea. There are still a great number of defects to be remedied, but providing interest and keenness in the specialty can be stimulated in our students and younger graduates, its modest success will continue.

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Does gonorrhoea surveillance pay?

ROBERT C. RENDTORFF AND HENRY PACKER

From the Departments of Community Medicine and Microbiology, University of Tennessee Center for the Health Sciences, and the Memphis-Shelby County Health Department, Memphis, Tennessee, USA

Introduction

In previous studies which emphasised the role of the urban community hospital in gonorrhoea surveillance, the effectiveness of the community-wide screening programme in reducing serious complications of the disease in women has been stressed (Rendtorff *et al.*, 1976, 1977). While the rate of gonorrhoea has remained essentially at the same level during the past four years, the incidence of pelvic inflammatory disease and salpingitis among patients admitted to the City of Memphis Hospital has shown a marked decline since the surveillance programme was set up in Memphis-Shelby County, Tennessee, in 1968.

'It is conceivable that a continuing surveillance programme for gonorrhoea in women, reaching a large segment of the population at risk, may have no impact upon the infection rate, which is primarily a behavioural problem, but it may nevertheless stop the infection from progressing to pelvic inflammatory disease by treating early infections' (Rendtorff *et al.*, 1977). This suggestion that a surveillance programme might be successful in reducing the incidence of complications of gonorrhoea in women prompted us to look further into the situation in Memphis.

Incidence of PID and salpingitis

There are four central city hospitals in Memphis, and data on the incidence of pelvic inflammatory disease and acute and chronic salpingitis were obtained from each hospital. These conditions are often gonococcal in origin, as previous studies have indicated (Rendtorff *et al.*, 1974). The number of such cases reported each year from the discharge diagnosis (disease index) for each hospital was divided by the annual number of discharges. These

data were then plotted as a three-year moving average of rates of cases per 1000 admissions as shown in the Figure. The biases inherent in these data, especially in the use of the denominator statistic, are well recognised; however, these should be fairly consistent over the years and not markedly affect the slopes of the curves.

Data from two hospitals, City of Memphis Hospital and hospital A, showed a marked decline in the incidence rate of these complications during the period of the study. Figures from two other hospitals, B and C, showed more constant rates for these conditions. To interpret these differences in the rates between the hospitals the characteristics of patients served by each hospital must be examined.

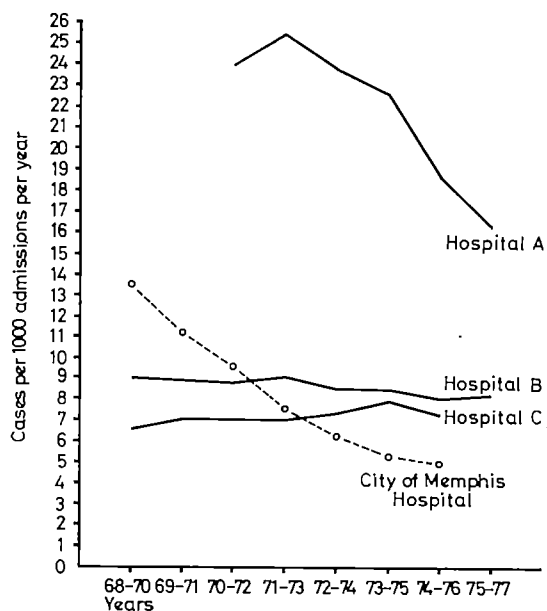


Figure Incidence rate of pelvic inflammatory disease and salpingitis in four Memphis hospitals (by three-year moving average)

Address for reprints: Dr R. C. Rendtorff, Department of Community Medicine, University of Tennessee, 800 Madison Avenue, Box GA150, Memphis, Tennessee 38163, USA

Received for publication 15 August 1978

POPULATION GROUPS

The City of Memphis Hospital serves the indigent poor of the city. Only 18% of its patients live outside the county, and these are from the surrounding metropolitan areas which cross state lines. Hospital A is located in a very low socioeconomic area of the city. Both hospitals serve patients from the indigent to lower socioeconomic levels, the women of which group are very likely to be screened in the surveillance programme, which is carried out intensively among this sector of the population. Statistics from both hospitals showed a marked decline in the rates of complications over the period of the study. The higher rates at hospital A may be due to a greater availability of beds and to its smaller size, which affects the denominator. This private hospital has an excess of beds available, and physicians practising here tend to admit their patients to hospital for treatment. In contrast, the City of Memphis Hospital is a larger, overcrowded university teaching hospital. Beds are at a premium and patients with common conditions, such as pelvic inflammatory disease and salpingitis, are frequently treated as outpatients rather than in hospital. A recent study has shown that only one out of 30 women with pelvic inflammatory disease seen in the city hospital emergency room was admitted.

The two private hospitals, B and C, serve an entirely different population of patients; these are of higher socioeconomic status and generally would not have participated in the surveillance screening programme. Of the patients at hospital C 57% are

referred from a seven-state medical catchment area beyond the metropolitan area. Hospital B similarly receives many of its patients by referral from the same area. These two hospitals in effect serve as a control, since their patients are not reached by the screening system and one would not expect the rate of complications to vary over the years.

Conclusion

The data given in the Figure show the beneficial effects of community-wide screening for gonorrhoea in women. A previous study on the costs of treating gonorrhoea in women has already stressed the vast economic consequences of this disease, not to mention its devastating effects on the lives of those infected (Rendtorff *et al.*, 1974). The social implications of gonorrhoea in this sense must be fully recognised. It is a truly devastating disease in women, and, economically, gonorrhoea surveillance is worthwhile.

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An alternative to the problem of insufficient physician manpower in the clinical care of sexually transmitted diseases

CHARLES W. PARTON*† AND SUZANNE M. SGROI*

*From the *Department of Ambulatory and Community Medicine, Mount Sinai Hospital, and the*

†Department of Community Medicine and Health Care, University of Connecticut Medical School, USA

Introduction

In defining the problem of disproportion in the provision of medical care, it is helpful to divide it into three main categories: geographic, socio-economic-cultural, and disease-related. The Hartford area in affluent Connecticut, as in other less fortunate areas, has significant distribution problems in each of these three categories. In this paper, however, I shall consider the last of these, the disease-related maldistribution of physician services.

Manpower deficiencies in STD clinics

Among the new trends in the epidemiology of sexually transmitted diseases it is pertinent to discuss not only the mechanisms and trends of disease processes but also some of the problems that interfere with the management of the disease.

Health departments at all levels in the United States often have difficulty in recruiting physicians with a sustained interest in running well-controlled, high-quality, comprehensive sexually transmitted disease (STD) clinics. Most physicians look on the problem of 'VD' with as much interest and enthusiasm as they do a back-ache.

The reasons for this attitude are many, but two stand out clearly. The health hazards of the various sexually transmitted diseases are not even partially realised by the practising physician and as yet little effort has been spent in our medical schools to change this.

The belief that syphilis is almost controlled and that its identification is assured by serological testing at premarital examinations and on admission to hospital, coupled with the dramatic response to treatment by penicillin, has rid our streets and our consciousness of the cardiovascular and neurological complications of the syphilis of the past. Furthermore, the ease with which a case of gonorrhoea is treated fails to alert the physician to the significance of the disease. It is our contention, based on several studies, that 65 000-100 000 young American women become sterile following gonococcal salpingitis each year. Because of the delay and confusion in relating these complications to the primary cause there is little concern among physicians and governments (Sgroi, 1978).

Other examples of inadequate service due to lack of, or suppression of, concern by physicians are: (1) the rising incidence of sexual molestation of children and the resulting STD problems with these children; and (2) the special needs of the male homosexual in whom 50% of cases of syphilis occur, but for whom society has little understanding.

The second major cause of poor physician distribution is the inadequate pay scale for health department physicians. While the dedicated public health service physician devotes himself or herself to high-quality and conscientious work, the local 'VD clinic physician' is frequently elderly, outmoded, uninterested, or just unobtainable.

Situation in Hartford, Connecticut, in 1975

The city of Hartford in central Connecticut, half-way between Boston and New York City, has an urban population of 158 000 people, about 40% of whom are black or of Hispanic ethnic origin. The city, whose average annual income is \$13 000 and 38% of whose population receives city or state welfare benefits, is the capital of one of the wealthiest

Address for reprints: Dr C. W. Parton, Department of Ambulatory and Community Medicine, Mount Sinai Hospital, 500 Blue Hills Avenue, Hartford, Connecticut 06112, USA

Received for publication 21 September 1978

states in the country with its aircraft and light industries, yankee ingenuity, and insurance underwriting. Indeed, Hartford is known as the 'insurance capital of the world'.

The physician-to-population ratio in the city is 1:614. There are the University of Connecticut Medical Dental School and three university affiliated community hospitals, and the quality and quantity of medical care is virtually unsurpassed. Nevertheless, in 1974 Hartford's health director was unable to employ a suitable physician to run the city health department's communicable disease clinic.

Since its creation by the Mount Sinai Hospital in 1972, the department of ambulatory and community medicine has advocated a systematic approach to meeting community health care needs, especially where there is maldistribution of physicians. It was thus natural that the hospital and the health department entered into a contractual relationship to help to solve this problem.

Hospital and city health department co-operation

The concept of a community hospital and health department co-operating in a co-ordinated programme is not well established. In negotiations between the City Health Director and the Chief of Community Medicine at the Mount Sinai Hospital the following elements were considered:

- (1) The need of the city for physician services.
- (2) The ability of the hospital's resources and constraints to meet these needs, based on the fact that (a) the need was real; (b) that there was neither competition with the private practice sector nor duplication of service; (c) the programme was financially feasible; (d) that the hospital could deliver quality care; and (e) that there would be benefits derived to the hospital, such as good public relations, patient referrals, and a challenge for department personnel.
- (3) The city's resources included: (a) salaries for the physician, nurse, and clerk; (b) a well-managed complete laboratory service; and (c) existing space and equipment.
- (4) The city's health department would retain its natural functions of providing services in epidemiology, education, and case-finding.
- (5) The state resources supplied most medication and some financing to the city.
- (6) The hospital would develop new sources of finance, by charging regular clinic fees to the city and state welfare department, where appropriate. It was noted that in no way must the inability, or even the lack of desire to pay,

be a deterrent to full and conscientious management.

The Mount Sinai Hospital department of ambulatory and community medicine, after surveying the needs, available resources, and financial mechanisms, developed the following programme.

PROGRAMME

Clinical management

The needs of the clinic demanded accurate diagnostic procedures, including in-depth interviewing covering all aspects of STD and all possible sources and routes of transmission of disease. In addition, adequate general and specific examinations were needed to rule out or to refer elsewhere other health problems which were not managed in the clinic. As regards treatment, the question when, or whether, to treat patients with and without obvious clinical manifestations had to be decided. Finally, appropriate management protocols were needed for each category of STD by age, sex, and special need.

Staff

In approaching the problem of maldistribution of physicians our department has developed pools of nurse practitioners, physician associates, and physicians, who rotate through various assignments, no one of which would necessarily be stimulating on a permanent basis but which together provide a varied and exciting medical challenge (Figure).

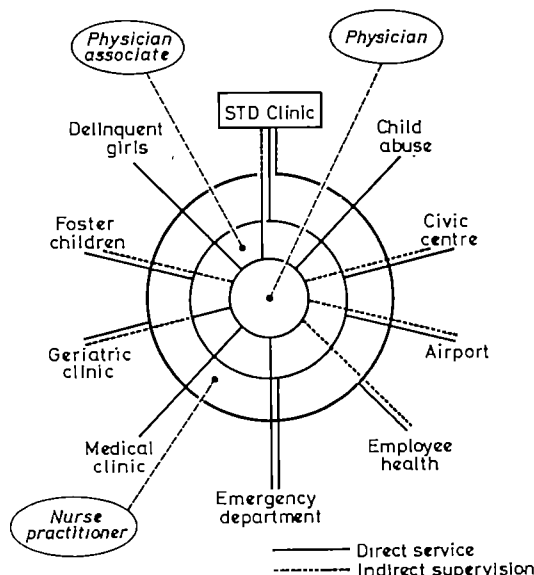


Figure Mount Sinai Hospital outreach programme

We believed that the physician associates, who are all college graduates with two years of accredited study as generalists and who are all certified by national board examinations, could provide a high level of skill at considerably lower cost; the availability of personnel was assured through rotation. There would also be a need for nurses in the clinic, but their role could also be expanded to provide equally capable diagnostic and treatment skills. In providing this service, however, we were responsible for the quality, reproducibility, and continuity of care. We planned, therefore, a specialised training in STD for these two groups.

Training in STD

Dr Suzanne Sgroi, our clinic director, having first developed a management protocol, undertook the design and production of a training course, which consisted of 15 hours of didactic lectures and reading assignments followed by 15 hours of directly supervised training in the clinic. This latter section, which follows successful completion of a written examination, provides preceptorial clinic experience followed by practical experience under direct supervision, so that the student not only sees how it is done but does it with continuous critique.

The hospital's clerical personnel, who have also participated in the educational programme, provide invaluable help in answering the telephone. They convey to callers our desire to help by their warmth and understanding.

Finance

The city health department has had no increase in costs over their pre-contract budget. There are now more employees providing more service to more patients than ever before. Clearly one source of savings has been the use of physician assistants and nurse practitioners in the clinic instead of physicians. The hospital now bills city and state insurance schemes, and is investigating direct collections for those patients who volunteer to pay for their service—which to our surprise was common. We made it abundantly clear that no patient will be denied treatment for lack of ability, or indeed lack of desire, to pay. So far we have had no complaint or loss of clientele.

Physician supervision

We were also concerned with physician supervision and audit of the quality of care. This component is provided by our physicians under the clinic director. These physicians attend in rotation for three hours each day and provide supervision in the clinic. They directly observe physician associates and nurse practitioners, and are consulted on specific problems; they carefully review each patient's case record for completeness and compliance with our protocol.

During the clinic hours when a physician is not present patients are diagnosed and treated by the physician assistant and nurse practitioner groups following the protocol. There is indirect physician supervision by telephone from a nearby primary care practice clinic or from the hospital emergency department.

In the past year one full-term equivalent (FTE) nurse practitioner and one FTE physician assistant from our pool of eight physician assistants, who rotate services, cared for 9000 patients at a unit treatment cost of less than \$10.00.

Conclusions

Our programme demonstrates that high-quality clinical management of STDs, including diagnosis, treatment, and education, can be delivered by physician extenders working under direct or indirect physician supervision and using a protocol. The programme demands a thorough education in disease processes, epidemiology, and treatment under close medical scrutiny.

We believe this care to be more thorough and delivered with greater enthusiasm, compassion, and interest than is available in some clinics in the USA, in which all the clinical care is provided by physicians.

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Results of routine screening for sexually transmitted diseases in Austria over a six-year period

D. FANTA,* W. GEBHART,* W. GROSS,† E. M. KOKOSCHKA,*
J. SÖLTZ-SZÖTS,* AND J. THURNER*

From the *Epidemiology Unit, Department of Dermatology II, University of Vienna, and the
†Department of Dermatology, Krankenanstalt Rudolfstiftung, Vienna, Austria‡

Introduction

The epidemiology of sexually transmitted disease (STD) changes rapidly with the increasingly rapid alterations in modern social behaviour and prevailing therapies, and with the changing biological activities of the causative agents. An important parameter in the control of STD is, therefore, an up-to-date knowledge of recent developments in this field. We have tried to analyse the situation in Vienna by pooling the results of screening of STDs, which, because of the local situation, is carried out by a rather heterogeneous group of medical institutions. General governmental care covers only small sections of the population and most of the patients are seen in hospitals, outpatient clinics, private referral laboratories, and private practice. Each of these institutions has its own special interest and its own, at least partly selected, patients.

Study material and results

In our study we collected the results of screening in four different groups of patients: (1) prostitutes under the health control of the governmental health service; (2) prostitutes who had been admitted to hospital for treatment of their STD; (3) outpatients of the second Viennese department of dermatology and venerology; and (4) patients sent to a microbiological laboratory by doctors in private practice.

LICENSED PROSTITUTES (GROUP 1)

Although the prostitutes comprise only a small group of patients they provide a useful indicator

of the epidemiology of STDs owing to the broad spectrum of their sexual activities. The licensed Viennese prostitutes are under strict health authority control and if they do not attend for their obligatory weekly test they lose their licence. The total number in this professional group has increased from 548 in 1971 to 570 in 1977, indicating a balanced state. Nevertheless, there is a marked fluctuation in the incidence of STDs (Table 1).

Based on approximately 30 000 tests a year the prostitutes' control authority found a significant decrease in the classical venereal diseases in this group. The incidence of gonorrhoea, which had reached a peak in 1971, when 199 out of 548 prostitutes had acquired the disease, decreased to 98 out of 570 cases among women in 1977. In 1971 only one case of syphilis was found but in 1974 and in 1977 the figures were 11 and two cases respectively.

Table 1 Number of cases of syphilis and gonorrhoea in licensed Viennese prostitutes (group 1)

	Year		
	1971	1974	1977
Gonorrhoea	199	175	98
Syphilis	1	11	2
Total no. of cases	548	556	570

HOSPITALISED PROSTITUTES (GROUP 2)

Since 1972, 1200 licensed as well as unlicensed amateur prostitutes with gonorrhoea have been admitted to hospital for repeated cultural, serological, cytological, and clinical investigations to evaluate the incidence of simultaneously existing STDs (Söltz-Szöts and Kokoschka, 1973) (Table 2).

Oral gonorrhoea does not seem to be a major problem among Viennese prostitutes, since only 8% of all patients with gonorrhoea (group 2) had positive pharyngeal culture results. An increase in the incidence of syphilis of from 1.5% to 5% was also observed in this group.

‡In co-operation with the Ludwig Boltzmann Institute for Research on Infectious Venereodermatological Diseases

Address for reprints: J. Söltz-Szöts, Allgemeines Krankenhaus des Stadt Wien, II Universitäts-Hautklinik, 9 Alserstrasse 4, A-1090 Vienna, Austria

Received for publication 20 November 1978

Table 2 *Other STDs occurring simultaneously in 1200 prostitutes with gonorrhoea (group 2)*

Other STDs	Year	
	1972/73 (%)	1976/77 (%)
Oral gonorrhoea	NK	8
Syphilis	1.5	5
Trichomoniasis	35	56
Candidosis	15.5	43
Condylomata acuminata	2.5	1.6
Pediculosis pubis	NK	1.5
Scabies	7.5	3.6

NK not known

A more pronounced increase was found in the incidence of trichomoniasis and candidosis between 1972 and 1977, although the same diagnostic procedures were applied. The incidence of trichomoniasis increased from 35% to 56% and that of candidosis from 15.5% to 43% in prostitutes with gonorrhoea in group 2. In contrast, the incidence of condylomata acuminata and scabies was again found to be lower in recent years than in 1972 and 1973 (Table 2).

OUTPATIENTS (GROUP 3)

In the STD unit of the second department of dermatology in Vienna, where in Austria mainly dermatological patients are seen, more than 12 000 patients with STDs attended for medical advice in the last six years (Table 3).

Gonorrhoea was detected in 21% of male and 16% of female patients in group 3; syphilis occurred in 11% and 7.5% respectively. The figures for trichomoniasis and candidosis differ greatly from those shown for the prostitutes, the reason for this being the non-random selection of patients referred to the clinic. Of all female patients 22% had candidosis and 9% trichomoniasis; the percentage in male patients was much lower at 6% and 2.5% respectively. Genital herpes was observed in 5.6% of the patients. Interestingly enough, chancroid was found in five cases in 1977 after a

Table 3 *Final diagnoses of 1200 patients (7500 male and 4500 female) attending the STD unit (group 3)*

Diagnosis	Men (%)	Women (%)
Gonorrhoea	21	16
Syphilis	11	7.5
Trichomoniasis	2.5	9
Candidosis	6	22
Herpes genitalis	5.6	1.5
Condylomata acuminata	9	5
Pediculosis	1.5	1
Scabies	9.5	14
Chancroid	0.06	

10-year period of complete absence in Vienna. So perhaps we will have to expect a new period of activity of this disease.

PRIVATE MICROBIOLOGICAL LABORATORY PATIENTS (GROUP 4)

Finally, 39 435 patients were seen in a private laboratory, to which they were referred for cultural diagnosis by dermatologists in private practice. Gonorrhoea was confirmed in 83.4% of the male and 76.7% of the female patients after the doctor had suspected this on clinical and microscopical grounds. On the other hand, only 0.6% and 1% of male and female patients respectively were found to have gonorrhoea when their doctor had suspected a non-specific STD. The additional information provided by culture tests confirmed only about 1.6% of cases after experienced investigators had made a diagnosis of gonorrhoea on the basis of the Gram-stained smears.

Trichomoniasis was detected in 8% of male and 14.8% of female patients with gonorrhoea and in 8.6% and 38% respectively when a non-specific STD was diagnosed by the practitioner. Candidosis predominantly occurred in female patients; 31% of female patients with a discharge and 5.8% of male patients had candidosis compared with no cases when gonorrhoea was suspected.

Discussion

In Vienna, as in other countries (Wallin, 1978), there has been a marked decrease in the number of cases of gonorrhoea since 1971. Although the same methods of investigation have been used the number of cases of condylomata acuminata, scabies, and genital herpes are at about the same level or have decreased slightly. Chancroid reappeared in Vienna in 1977 after a long period of absence.

From these trends in Vienna, we conclude that efforts to control gonorrhoea and syphilis have been successful and should be continued. More attention, however, should be directed towards measures against the increasing incidence of trichomoniasis and candidosis. In addition, some rare venereal diseases—such as chancroid—might reappear locally in the near future. These conditions should, therefore, be included in the training of doctors and students.

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Epidemiological importance of concealed nongonococcal urethritis

R. R. WILLCOX

From the Department of Venereology, St Mary's Hospital, London, and King Edward VII Hospital, Windsor

SUMMARY Of the 229 806 new male patients attending venereal disease clinics in England in 1976, 31.9% had recognisable non-specific genital infection (NSGI), which was easily the most common diagnostic category of the sexually transmitted diseases (STDs) and one which had increased more markedly than all the others in the previous 25 years. Many more cases of nongonococcal urethritis (NGU) are concealed under the diagnosis of gonorrhoea, as is clear from the high incidence of post-gonococcal infection following treatment with penicillin. From 44% to 56% of male patients with gonorrhoea can also have non-specific genital infection during the same year. A practical treatment schedule for gonorrhoea which is also effective against simultaneously acquired non-gonococcal urethritis would be of great value.

Introduction

Nongonococcal urethritis (NGU) is one of the greatest problems in the management of the sexually transmitted diseases (STD) and represents a source of much psychological trauma to those affected. It is by far the most common condition requiring treatment in British clinics.

Epidemiological data

RELATIVE IMPORTANCE OF THE STDs IN MEN
The relative importance of the recorded sexually transmitted diseases in men in the clinics in England is outlined in Table 1; the dominant role of non-specific genital infection (NSGI) is clear. In 1976 the number of cases of NSGI in men was virtually twice that of gonorrhoea, five times that of venereal warts, 11 times that of candidosis, 16-19 times that of herpes simplex or pediculosis pubis, 25 times that of syphilis, 38-44 times that of scabies and trichomoniasis, no less than 123 times that of molluscum contagiosum, and 727 times that of chancroid, lymphogranuloma venereum, and granuloma inguinale combined.

It should be noted that the figures for NSGI now include some patients with non-specific proctitis: the vast majority of cases are, nevertheless, of nongonococcal urethritis.

Table 1 *Relative importance of the sexually transmitted diseases in men (England 1976)*

Diseases	No. of cases	Relative incidence %
Non-specific genital infection	73 425	100
Post-pubertal gonorrhoea	37 179	50.6
Genital warts	14 389	19.6
Candidosis	6601	9.0
Herpes simplex	4458	6.1
Pediculosis pubis	3905	5.3
Syphilis (total)	2999	4.1
Scabies	1937	2.6
Trichomoniasis	1682	2.3
Molluscum contagiosum	598	0.8
Chancroid, lymphogranuloma venereum, and granuloma inguinale combined	101	0.1

PROPORTION OF CASES OF NSGI

Grouped figures for new cases of STD in men attending sexually transmitted disease clinics in England in 1976 are shown in Table 2.

In 1976, of the 229 806 male patients attending clinics in England with a new diagnosis, 25% were not treated at all or were given treatment for treponemal disease or gonorrhoea which was definitely not sexually transmitted (Table 2). Of the remainder, 15.9% had sexually transmitted diseases other than gonorrhoea or non-specific genital infection, 10.9% were treated for other conditions, which included anything from tinea cruris to those conditions treated epidemiologically in the absence of any evidence of disease, 16.2% had post-pubertal gonorrhoea, while no less than 31.9% had NSGI.

Address for reprints: Dr R. R. Willcox, 'Tideway', Lonsdale Road London SW13

Received for publication 8 June 1978

Table 2 *Relative proportion of non-specific genital infection (England 1976)*

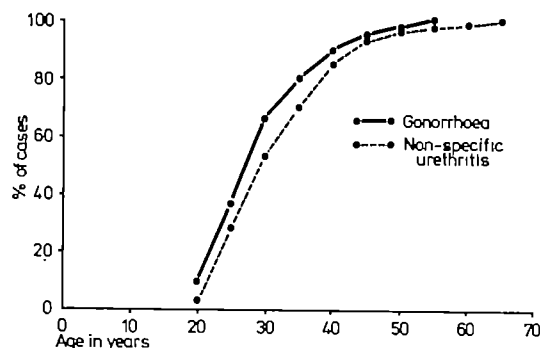
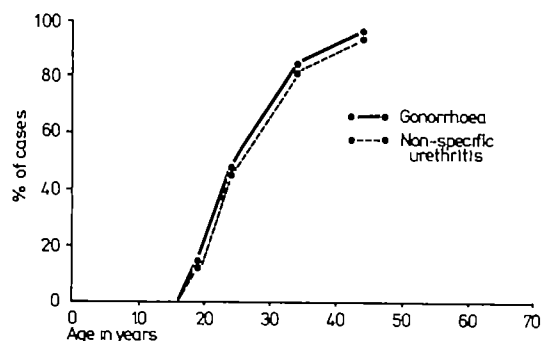
Condition	No. of cases	% of total	% at highest risk
Non-specific genital infection*	73 425	31.9	42.6
Post-pubertal gonorrhoea	37 179	16.2	21.6
Other STDs†	36 622	15.9	21.2
Other conditions (treated)	25 139	10.9	14.6
Total no. of cases at highest risk	172 365	75.0	100.0
Other conditions (not treated)‡	57 441	25.0	
Total	229 806	100.0	

*Including those with arthritis
 †including those with acquired syphilis
 ‡including all other patients receiving no treatment or referred elsewhere (48 with congenital syphilis, 710 treated for other treponemal disease, and nine with pre-pubertal gonorrhoea). The total number receiving treatment was thus 173 132.

If the 25% of patients with other conditions, who were seen mainly because of anxiety and who were given no treatment, are excluded, however, the 172 365 new patients remaining represent those at highest risk. Of these, 14.6% were treated for conditions other than STDs, 21.2% were treated for a sexually transmitted disease other than syphilis or gonorrhoea, while 21.6% had post-pubertal gonorrhoea, and no less than 42.6% were classified as having NSGI.

AGE DISTRIBUTION

While national data on the age groups of patients with primary and secondary syphilis and with gonorrhoea are collected, no data are assembled centrally for the remaining sexually transmitted diseases. At the STD clinics both at St Mary's and Windsor Hospitals nongonococcal urethritis affected a slightly older age group in men than did gonorrhoea (Figs. 1 and 2).

Fig. 1 *Age distribution of male cases of gonorrhoea and non-specific urethritis at St Mary's Hospital, London, in 1952*Fig. 2 *Age distribution of male cases of gonorrhoea and non-specific urethritis at Windsor Hospital in 1977*

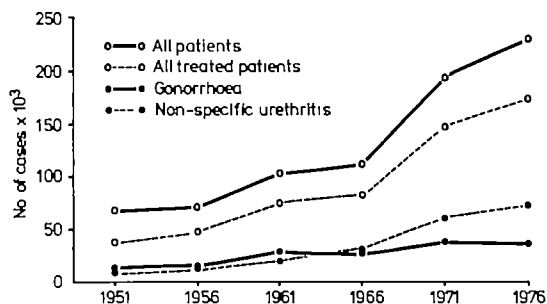
INCIDENCE OF NSGI (1951-76)

In the period from 1951 to 1976, the relative increase in the incidence of NSU or NSGI in men in the clinics (Table 3) was markedly higher than that of gonorrhoea or of patients requiring treatment for conditions other than urethritis (Fig. 3).

The 1951 and 1976 figures are not quite comparable (Table 3); those of 1976 included all forms of non-specific genital infection, including non-specific proctitis, which tends therefore to increase the more recent figures, as proctitis was not included

Table 3 *Relative increase in incidence of NSGI*

Condition	1951 (England and Wales)	1976 (England only)	Multiple of 1978/1951
New patients	69 275	229 806	× 3.3
Treated patients	39 319	172 365	× 4.4
With gonorrhoea	14 975	37 179	× 2.5
Treated for conditions other than urethritis	13 550	61 761	× 4.6
With NSGI	10 794	73 425	× 6.8

Fig. 3 *Number of male cases of gonorrhoea and non-specific urethritis in STD clinics in the United Kingdom from 1951 to 1976*

in 1951. This is offset by the fact that the figures quoted for 1951 refer to England and Wales, while those for 1976 (as a result of devolutionary pressures) only refer to England.

Thus with these provisions the number of male patients with gonorrhoea increased approximately 2.5 times, the number of patients treated for conditions other than urethritis increased 4.6 times, while the number of male patients with non-specific genital infections increased by at least 6.8 times.

INCIDENCE OF CONCEALED NSU

While some cases of gonorrhoea will be concealed under the diagnosis of 'other conditions requiring treatment' (for example, the relatively few men given epidemiological treatment after only one test to exclude gonorrhoea) and under the diagnosis of non-specific genital infection in clinics where cultures as well as Gram-stained smears are not routinely made on male patients, most cases of concealed gonorrhoea in men offer no great community problem as these patients have usually either been given epidemiological treatment or treated with tetracycline for nongonococcal urethritis—which treatment in practice is usually also effective against gonorrhoea.

Double infections are much more important among cases of gonorrhoea, as the treatment given for gonorrhoea (for example, penicillin or ampicillin) may well be ineffective against simultaneously acquired non-specific urethritis. This is evident from the fact that high rates of post-gonococcal, nongonococcal urethritis are noted in some clinics especially after treatment with the penicillins (Table 4).

Although some cases may be due to a persistence of inflammation with resolving gonorrhoea these are thought in the main to result from double infections. Those infections diagnosed in men are likely subsequently to be treated with tetracycline, but this treatment will not cover either non-specific infection in those who default or in those in whom the infection is subclinical or non-specific infections in female contacts of men with gonorrhoea.

Table 4 *Post-gonococcal, nongonococcal urethritis (St Mary's Hospital)*

Antibiotic (plus probenecid)	No. of patients		
	Treated	Retreated for NGU within two weeks	
		No.	%
Procaine penicillin	166	48	28.9
Ampicillin	353	95	28.5

ESTIMATED INCIDENCE OF DOUBLE INFECTIONS

It is difficult to estimate the exact incidence of double infections. Calculations can, nevertheless, be made of the proportion of nongonococcal infections in patients without gonorrhoea and this percentage can be applied to those who have gonorrhoea.

In 1976, of the 229 806 new patients seen in the clinics, 173 132 were given some treatment, and these represent the group at highest risk (Table 3). The size of the group at highest risk by these criteria is shown in Table 5.

The number of patients treated, with gonorrhoea and without gonorrhoea, are shown in Table 6. The proportion of treated male patients without gonorrhoea who had NSGI are shown in Table 7 (Fig. 4); this increased from 44.3% in 1951 to 54% in 1976.

Table 5 *Total number of new patients excluding those given no treatment*

Year	Total no. of new patients	Untreated	Treated
1951	69 275	29 956	39 319
1956	72 055	23 514	48 541
1961	103 077	27 567	75 510
1966	112 926	29 414	83 512
1971	192 953	45 967	146 986
1976	229 806	56 674	173 132
% rise (1951-76)	231.7	189.2	340.3

Table 6 *Total number of new, treated patients with and without gonorrhoea*

Year	No. of new patients		
	Given some treatment	With gonorrhoea	Without gonorrhoea
1951	39 319	14 975	25 344
1956	48 541	16 377	32 164
1961	75 510	29 519	45 991
1966	83 512	27 921	55 591
1971	146 986	39 055	107 931
1976	173 132	37 188	135 944

Table 7 *Proportion of treated patients without gonorrhoea with non-specific genital infection (1971 and 1976 England only)*

Year	Total no. of treated patients without gonorrhoea	Non-specific genital infection	
		No.	%
1951	25 344	10 794	44.3
1956	32 164	14 852	46.2
1961	45 991	21 472	46.7
1966	55 591	30 462	54.8
1971	107 931	60 708	56.2
1976	135 944	73 425	54.0

It can then be argued that the proportion of patients with gonorrhoea who have NSGI will be the same as the proportion of patients with a treated condition who have NSGI. The number of patients with gonorrhoea plus NSGI are calculated in Table 8 (Fig. 5).

Thus, in 1976, when there were 73 425 cases of symptomatic NSGI in men treated in the clinics an estimated additional 20 081 cases were concealed under the diagnosis of gonorrhoea, thus giving a total of over 93 000 cases quite apart from the

considerable numbers treated in private practice and the unknown thousands with asymptomatic infections.

EPIDEMIOLOGICAL IMPORTANCE OF DOUBLE INFECTIONS

If up to 50% or more of male patients with gonorrhoea carry the infectious agent of nongonococcal urethritis the epidemiological importance of double infections would be considerable.

The usual treatment given for gonorrhoea (such as, procaine penicillin, ampicillin, or spectinomycin) in the doses generally used is unlikely to suppress asymptomatic infections in patients of either sex. Even if treatment with tetracycline or erythromycin is given to men with post-gonococcal urethritis it is possible that this information will not be transmitted to the physician who may have already treated the female contact for gonorrhoea, particularly if this occurred at another unit or in private practice.

Conclusions

Non-specific genital infection is by far the most common diagnostic category for male patients attending venereal disease clinics in England. In 1976 the number of patients with this condition was virtually twice as many as those with gonorrhoea, five times those with venereal warts, and 25 times those with all forms of syphilis.

Of the 229 806 new patients seen in STD clinics in England in 1976 31.9% had recognisable NSGI. If the 25% of patients not receiving treatment, or who were treated for an asexually acquired gonococcal or treponemal infection, are excluded overt NSGI comprised no less than 42.6% of those cases at highest risk.

In the STD clinics during the 25 years from 1951 to 1976 the total number of new patients receiving treatment for gonorrhoea increased 2.5 times, those given treatment for conditions other than urethritis 4.6 times, while the number of patients with NSGI increased by no less than 6.8 times.

A significant amount of NSGI is known to be subclinical in both sexes. A number of cases too are concealed under the diagnosis of gonorrhoea, which can usually be expected to become apparent before simultaneously acquired nongonococcal urethritis. A probable 20%-30% of male patients treated for gonorrhoea with penicillins may subsequently require treatment for post-gonococcal, nongonococcal urethritis. These figures represent manifest cases; other patients with gonorrhoea (and their consorts) must also carry an asymptomatic infection.

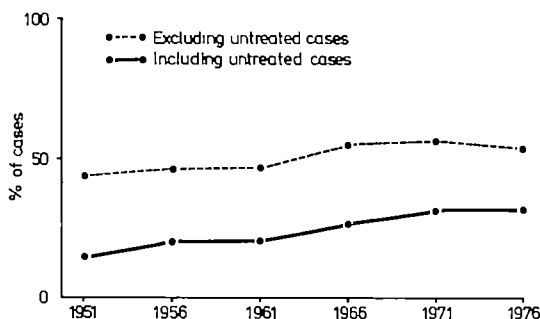


Fig. 4 Percentage of patients without gonorrhoea with non-specific urethritis

Table 8 Estimated number of patients with double infections (England and Wales)

Year	Total no. of men with gonorrhoea	Double infections	
		No.	%
1951	14 975	6634	44.3
1956	16 377	7566	46.2
1961	29 519	17 785	46.7
1966	27 921	15 300	54.8
1971*	39 055	21 949	56.2
1976*	37 188	20 081	54.0

*England only

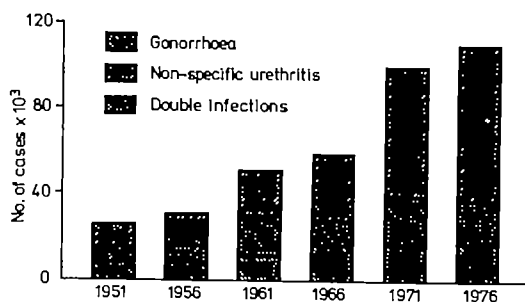


Fig. 5 Estimated number of patients with gonorrhoea with non-specific urethritis

An attempt has been made to estimate the incidence of NSGI among male patients treated for gonorrhoea. Those patients given no treatment were first excluded and those treated for any condition with or without urethritis were taken to be those at highest risk. From these data calculations were made of the incidence of urethritis among patients with sexually transmitted diseases but without gonorrhoea which was then applied to those with gonorrhoea.

Even though the data on which the calculations are based are annual figures, it appears nevertheless that 44%–56% of patients with gonorrhoea may have NSGI in the same year, mostly as a direct consequence of the exposure that led to the gonorrhoea.

The figures suggest that the epidemiological importance of concealed non-specific genital infection is numerically very significant. The possible

ultimate control of this disease by means of diagnosis and treatment must therefore be considered doubtful. As a starting point, however, it would be desirable to formulate a treatment schedule which is effective against both gonorrhoea and NSGI for patients with clinical manifestations as well as their consorts. This treatment should be competitive in price with the regimens currently used for gonorrhoea in developing and developed countries (without any increase in anticipated side effects) and should be capable of being given in a single dose, or at most two doses. No product which will meet these specifications is currently available.

Thanks are expressed to Dr M. Sibellas of the Department of Health and Social Security for kindly providing the 1976 clinic figures used in the calculations.

Abstracts

These selected abstracts and titles from the world literature are arranged in the following sections:

Syphilis and other treponematoses

(Clinical and therapy; serology and biological false-positive phenomenon; pathology and experimental)

Gonorrhoea

(Clinical; microbiology; therapy)

Non-specific genital infection

Reiter's disease

Trichomoniasis

Candidosis

Genital herpes

Other sexually transmitted diseases

Public health and social aspects

Miscellaneous

Syphilis and other treponematoses (Clinical and therapy)

C-reactive protein, C3, C4 and properdin during the Jarisch-Herxheimer reaction in early syphilis

C.-O. KINDMARK, H. MOLLER, AND K. PERSSON (1978).

Acta Medica Scandinavica, 204, 287-290

The occurrence of plasma CRP during the febrile response after the first injection of penicillin was followed in 10 patients with early syphilis. An increase in CRP was noted after 12 hours, with a maximum after 24 hours. The appearance of this acute phase protein could not be correlated to cutaneous reaction, increased body temperature, or leucocytosis, nor were baseline values of CRP correlated to clinical or serological activity of the infection. Patients with high levels of CRP before treatment suffered the most intense systemic reactions. No activation of the complement system during the Jarisch-Herxheimer reaction was detected from sequential determinations of C3, C4, and properdin.

Authors' summary

Syphilis (Serology and biological false-positive phenomenon)

Heated versus unheated sera in a microhemagglutination assay for antibodies to *Treponema pallidum*

S. A. LARSEN, D. C. CRUCE, C. E. FARSHY, AND J. C. FEELEY (1978). *Journal of Clinical Microbiology*, 8, 468

Syphilis (Pathology and experimental)

Detection by direct immunofluorescence of antibodies to *Treponema pallidum* in the cutaneous infiltrates of rabbit syphilomas

K. SOLTANI, I. K. ARONSON, F. BRICKMAN, AND A. L. LORINCZ (1978). *Journal of Infectious Diseases*, 138, 222-226

By direct immunofluorescence, with use of fluorescein-labelled sonified *Treponema pallidum*, specific antibodies were detected in the tissue infiltrates of cutaneous syphilomas in rabbits. Specimens from cutaneous and mucosal inflammatory lesions induced by intradermal injection of a keratinous substance from an epidermal cyst served as controls. Granular fluorescence was detected in the dermis of 11 of the 12 syphiloma specimens and corresponded to areas of heavy plasma cell infiltrates, and some fluorescence was found directly on plasma cells identified by subsequent staining with haematoxylin and eosin. This fluorescence could be blocked by preabsorption. Control slides did not show any fluorescence. Immunopathological techniques using labelled antigens may be of diagnostic value in syphilis and other infectious disorders which feature specific infiltrates.

Authors' summary

Respiration and oxidative phosphorylation in *Treponema pallidum*

P. G. LYSKO AND C. D. COX (1978). *Infection and Immunity*, 21, 462-466

Radiolabelling of *Treponema pallidum* (Nichols virulent strain) in vitro with precursors for protein and RNA biosynthesis

P. L. SANDOK AND H. M. JENKIN (1978). *Infection and Immunity*, 22, 22-32

Gonorrhoea (Clinical)

Gonorrhea detection in posthysterectomy patients

B. D. KLAUS, J. E. CHANDLER, AND P. E. DANS (1978). *Journal of the American Medical Association*, 240, 1360-1361

A retrospective study of women examined at a venereal disease clinic after hysterectomy was made to determine which culture site was most likely to show anogenital *Neisseria gonorrhoeae* infection when the cervix was absent. Seventeen of 104 such patients were culture-positive for *N. gonorrhoeae*. Rates of positivity for our culture sites were as follows: urethral, 17/17 (100%); vaginal, 7/17 (41%); and rectal, 2/17 (12%).

Authors' summary

Perihepatitis associated with salpingitis in adolescents

I. F. LITT AND M. I. COHEN (1978). *Journal of the American Medical Association*, 240, 1253-1254

Perihepatitis, one manifestation of complicated gonorrhoea, is infrequently diagnosed and reported. This study was undertaken to examine the incidence of gonococcal perihepatitis in adolescents as well as to study the effect, if any, of this condition on tests of liver function.

Accordingly, the records of 137 adolescents with salpingitis were evaluated. Right upper quadrant tenderness or hepatic enlargement was noted in 27, while raised aminotransferase levels were observed in 19 of the 59 in whom these tests were performed. All findings resolved promptly following initiation of intravenous penicillin therapy. The observation of hepatic dysfunction in 27% of these adolescents suggests that perihepatitis occurs more frequently than reported in adults and should be considered in the evaluation of the conditions of adolescents with salpingitis or upper abdominal tenderness.

Authors' summary

Asymptomatic gonococcal urethritis in selected males

E. H. BRAFF AND C. J. WIBBELSMAN (1978). *American Journal of Public Health*, 68, 779-780

Gonorrhoea in homosexual men

T. W. AUSTIN, B. LENT, AND F. L. M. PATTISON (1978). *Canadian Medical Association Journal*, 119, 733-739

Neonatal gonococcal orogastric contamination (case report)

C. CARBONETTO (1978). *Journal of the American Medical Association*, 240, 861

Gonorrhoea and non-gonococcal urethritis—recent advances

H. H. HANDSFIELD (1978). *Medical Clinics of North America*, 62, 925-945

Gonorrhoea (Microbiology)

Phenotypic and epidemiologic correlates of auxotype in *Neisseria gonorrhoeae*

J. S. KNAPP, C. THORNSBERRY, G. A. SCHOOLNIK, P. J. WIESNER, K. K. HOLMES *et al.* (1978). *Journal of Infectious Diseases*, 138, 160-165

Previous studies from Seattle, Washington, suggested that strains of *Neisseria gonorrhoeae* which require arginine, hypoxanthine, and uracil (Arg-Hyx-Ura-auxo-type) are uniformly highly susceptible to penicillin G, are relatively resistant to complement-dependent killing by heated, pooled, human serum, and are associated with disseminated gonococcal infection.

For further study of the epidemiology of these strains and for analysis of the susceptibility to penicillin, serum sensitivity, and the nutritional requirements of gonococcal isolates from other cities, a survey was made of urethral and cervical strains isolated in 1972 to 1974 from 50 randomly selected patients with uncomplicated gonorrhoea from each of nine cities. Arg-Hyx-Ura-strains represented >50% of isolates from Seattle and Des Moines, Iowa, 22% of isolates from Denver (Colorado), and Dayton (Ohio) and $\leq 12\%$ of the isolates from Boston (Massachusetts), Newark (New Jersey), Norfolk (Virginia), Miami (Florida), and Oakland (California). Arg-Hyx-Ura-strains were recovered from 42% of white and 9% of black patients ($P < 0.001$), and clinics with the highest incidences of these strains had the highest proportion of white patients among those with gonorrhoea. Arg-Hyx-Ura-strains were all susceptible to $\leq 0.125 \mu\text{g}$ of penicillin G/ml and were more resistant than strains with other auxotypes to killing by heat-inactivated human serum plus complement ($P < 0.01$).

Authors' summary

Effect of a staphylococcal on *Neisseria gonorrhoeae*

D. M. MORRIS, J. W. LAWSON, AND M. ROGOLSKY (1978). *Antimicrobial Agents and Chemotherapy*, 14, 218-223

Phage-group 2 staphylococcal strain UT0002 contains a large 56S virulence plasmid with genes that code for both exfoliative toxin and a specific staphylococcal termed Bac R₁. Four penicillinase-producing strains and three penicillin-susceptible strains of *Neisseria gonorrhoeae* were killed by Bac R₁. After 30 minutes of growth of the penicillin-resistant TR1 strain in 62.5 arbitrary units of Bac R₁ per ml, loss of viability was approximately 90%, and, after five hours, an approximately 99.99% loss of viability was observed. Lysis did not accompany cell death, and 84% of the Bac R₁ added to the growth medium was adsorbed to the gonococcal cells. The extracellular supernatant fluid from a substrain of staphylococcal strain UT0002 cured of the plasmid for Bac R₁ production had no lethal effect on the gonococcal strains. Bac R₁ was also shown to have bactericidal activity against an L-form of *Neisseria meningitidis*, indicating that the outer envelope of a neisserial cell is not needed

for bacteriocin activity. Ten different normal human sera were unable to neutralise Bac R₁ activity. The bacteriocin lacks adsorption specificity. It binds to but does not kill *Escherichia coli* cells, indicating that the cell envelope of Gram-negative organisms can provide protection against the staphylococcal.

Authors' summary

Effects of iron and culture filtrates on killing of *Neisseria gonorrhoeae* by normal human serum

P. NORROD AND R. P. WILLIAMS (1978). *Infection and Immunity*, 21, 918-924

Neisseria gonorrhoeae GC9, both colony types T2 and T4, were killed by normal human serum, although populations of colony type T4 were more susceptible. Ferric ammonium citrate prevented the killing of populations of both T2 and T4 colony types. Other iron compounds tested showed no protective effect, nor did ammonium citrate or the divalent cations magnesium or calcium. A filtrate from cultures of a *Neisseria gonorrhoeae* strain grown in a liquid defined medium showed a similar protective effect in the serum assay. The filtrate appeared to chelate iron, as measured by decreased ability of iron-free transferrin to bind iron in the presence of the filtrate. However, the two effects did not appear to be related. Neither ferric ammonium citrate nor the culture-free filtrate sufficiently inactivated complement to account for protection.

Authors' summary

Fluorescent antibody technique in identification of *Neisseria gonorrhoeae*—microcolonies grown on membrane filters

H. BLENK, W. JUNGE, AND B. BLENK (1978). *Zentralblatt für Bakteriologie*, 240, 480-489

Identification of *Neisseria gonorrhoeae* from primary cultures by a slide agglutination test

C. MALYSHEFF, R. WALLACE, F. E. ASHTON, B. B. DIENA, AND M. B. PERRY (1978). *Journal of Clinical Microbiology*, 8, 260-261

Antibody-cleaving neisseriae (leading article) *New England Journal of Medicine*, 1978, 299, 1011

Gonorrhoea (Therapy)

Susceptibility of *Neisseria gonorrhoeae* to cefoxitin sodium

I. PHILLIPS (1978).

Journal of Antimicrobial Chemotherapy, 4 (Suppl. B), 61-64

Minimum inhibitory concentrations (MICs) of cefoxitin sodium and benzylpenicillin for 48 gonococci were compared. Although benzylpenicillin was much more active on sensitive strains, the two agents similarly inhibited less sensitive strains at a concentration of about 1 mg/l. Cefoxitin activity was not affected by the production of β -lactamase by 5 β -lactamase-producing gonococci isolated in the UK and the USA, but cephaloridine, cephalothin, cephalixin, cephadrine, cefamandole, cefazolin, and cefuroxime were all less active against large than against small inocula. Despite this, cefuroxime was, weight for weight, more active than cefoxitin on large inocula.

Author's summary

Antibiotic susceptibility of *Neisseria gonorrhoeae* isolated in Johannesburg

R. M. ROBINSON-BROWNE, M. C. GAILLARD, H. J. KOORNHOF, AND J. SCHNEIDER (1978). *South African Medical Journal*, 54, 601-604

Non-specific genital infection

Effect of cortisol on the growth of *Chlamydia trachomatis* in McCoy cells

A. C. BUSHILL AND D. HOBSON (1978).

Infection and Immunity, 21, 946-953

The number of intracytoplasmic inclusions of *Chlamydia trachomatis* produced in McCoy cell monolayer cultures infected with a constant inoculum of a recently isolated genital strain was compared in cultures of untreated replicating cells and in monolayers which had been incubated in the presence of cortisol at initial extracellular concentrations between 0.0001 and 100 μ g/ml. The effect of adding cortisol was dependent on its concentration, on the time of addition to the tissue culture medium, and on the initial number of McCoy cells seeded to form the monolayer. When a concentration of 1.0 μ g/ml was added at the time of infection with *C. trachomatis*, the number of inclusions

detectable after a further 48 hours of incubation was increased by 1.84-fold over those detected in untreated cells. The mean size of inclusions and the ease of their recognition in McCoy cell cultures was also increased by this procedure.

Authors' summary

Prediction of efficacy of antimicrobial agents in treatment of infections due to *Chlamydia trachomatis*

W. R. BOWIE, C. K. LEE AND

E. R. ALEXANDER (1978). *Journal of Infectious Diseases*, 138, 655-659

Although *Chlamydia trachomatis* is readily eradicated by systemic therapy in patients with acute urethritis, systemic therapy is less satisfactory in treatment of chronic trachoma. The activities of antimicrobial agents against *C. trachomatis* in cell cultures when the antimicrobial agents are added one hour after the *C. trachomatis* (minimum inhibitory concentration [MIC]) predicts efficacy of the drugs in the treatment of urethritis but does not necessarily predict efficacy in the treatment of chronic ocular trachoma. Concentrations of antimicrobial agents required to eradicate *C. trachomatis* when the agents were added 48 hours after inoculation of the cell cultures with *C. trachomatis* exceeded the MIC by several logarithms, and minocycline, doxycycline, and rifampin were markedly more active than tetracycline, erythromycin, or several other antimicrobial agents. Of the three most active antimicrobial agents, only doxycycline has been used systemically to treat ocular infections due to *C. trachomatis*, and it has been reported to be the most effective antimicrobial agent that has been used. *In-vitro* testing of obligate intracellular pathogens, such as *C. trachomatis*, presents unique problems. The use of several methods of testing may help to identify antimicrobial agents with improved clinical efficacy, particularly in the treatment of ocular trachoma.

Authors' summary

In-vitro susceptibility of 30 strains of *Chlamydia trachomatis* to rosamicin

T. F. SMITH AND H. E. WASHTON (1978).

Antimicrobial Agents and Chemotherapy, 14, 493-494

A total of 13 of 30 clinical isolates of *Chlamydia trachomatis* were susceptible

in vitro to 0.01 μ g of rosamicin per ml. Only two of these strains were susceptible to tetracycline or erythromycin at this level. The results suggest that rosamicin may be useful for the treatment of chlamydial urethritis.

Authors' summary

Rapid serological test for diagnosis of chlamydial ocular infections

S. DAROUGAR, J. D. TREHARNE,

D. MINASSIAN, H. EL-SHEIKH, R. J. DINES, AND B. R. JONES (1978). *British Journal of Ophthalmology*, 62, 503-508

A rapid serodiagnostic test for the diagnosis of paratrachoma (TRIC ophthalmia neonatorum, inclusion conjunctivitis, TRIC punctate keratoconjunctivitis, and trachoma of sexually transmitted origin) has been developed. The technique is based on using a modified micro-immunofluorescence test for detecting antichlamydial IgG and IgM in the blood and IgG and IgA in tears. The blood samples are collected on cellulose sponges after a finger prick, and tears are collected by introducing small sponges into the lower conjunctival fornix of the eye. The blood and tear samples collected in this way could be sent to the diagnostic laboratory by post without special arrangements for cold storage.

In general the presence of antichlamydial IgG at a level of $>1/32$ or IgM at a level of $>1/8$ in blood and antichlamydial IgG or IgA at a level of $>1/8$ in tears was closely associated with ocular paratrachoma. The combined results of the micro-IF test of blood and tears has yielded the highest rate of positivity (80%). In patients with acute, untreated, paratrachoma the sensitivity of this test was similar to that of irradiated McCoy cells. In patients with a milder infection receiving antibiotics the sensitivity of the serodiagnostic test was superior to that of the cultural test. The high sensitivity and specificity of this rapid, simple, and inexpensive serodiagnostic test for the diagnosis of chlamydial ocular infections, coupled with simple and practical methods of collection and transport of blood and tear specimens, offer advantages over cultural tests for routine diagnosis and study of chlamydial ocular infections.

Authors' summary

Significance of appropriate techniques and media for isolation and identification of *Ureaplasma urealyticum* from clinical specimens

R. B. KUNDSEN, A. PARRENO, AND S. POULIN (1978). *Journal of Clinical Microbiology*, 8, 445-453

Controversy over the association of *Ureaplasma urealyticum* with reproductive failure may be due to methods used to isolate the micro-organism. *U. urealyticum* isolations from clinical material should be done simultaneously in broth and on Shepard's differential agar medium (A7) containing manganese sulphate. Urine sediments result in a 9% ($P=0.0002$) higher rate of isolation than cervical and urethral swabs. Primary isolations may not display standard textbook morphology. Isolated colonies may be present, but brown streaks in cervical mucus or a coalescent haze around epithelial cells in urine sediment may also be seen in areas of concentrated growth. The broth and agar media used, method of incubation, type of specimen, and method of storing specimens before culture are all factors which influence the recovery of *U. urealyticum*.

Authors' summary

Sensitivity of immunoperoxidase and immunofluorescence staining for detecting chlamydiae in conjunctival scrapings and in cell culture

R. M. WOODLAND, H. EL-SHEIKH, S. DAROUGAR, AND S. SQUIRES (1978). *Journal of Clinical Pathology*, 31, 1073-1077

Antichlamydial antibody in tears and sera, and serotypes of *Chlamydia trachomatis* isolated from schoolchildren in southern Tunisia

J. D. TREHARNE, R. ST C. DWYER, S. DAROUGAR, B. R. JONES, AND T. DAGHFOUS (1978). *British Journal of Ophthalmology*, 62, 509-515

Mycoplasma infection in pregnant rats: low viability of fetuses and newborn offspring

Y. NAOT, M. SHARF, AND A. KLEIN (1978). *Journal of Medical Microbiology*, 11, 261-267

Candidosis

Disc agar diffusion susceptibility testing of yeasts

M. A. SAUBOLLE AND P. D. HOEPRICH (1978). *Antimicrobial Agents and Chemotherapy*, 14, 517-530

A disc agar diffusion method was developed for testing the susceptibility of rapidly growing yeasts *in vitro*. A totally defined, completely synthetic agar culture medium (synthetic amino acid medium, fungal) and clinical isolates of *Candida* spp. and *Torulopsis glabrata* were used. Turbidimetric adjustment of cell suspensions resulted in standard, reproducible inocula, which gave sharp, clear zones of inhibition when applied by an agar overlay method. Optimal disc loads were determined for amphotericin B, amphotericin B methyl ester, 5-fluorocytosine, clotrimazole, and miconazole. Disc potencies were stable over a two-month period when stored in a vacuum desiccator at -30°C . Using an error rate-bounded classification, the zones of inhibition were correlated with both broth dilution and agar dilution minimum inhibitory concentrations (MICs). With amphotericin B and amphotericin B methyl ester, all isolates were susceptible, yielding zone diameters which clustered within 5 mm. Overall correlations between zone diameters and broth dilution MICs with 5-fluorocytosine, miconazole, and clotrimazole were 97, 96, and 82% (excluding *T. glabrata*) respectively; correlations of zone diameters with agar dilution MICs were 96, 92, and 88% respectively. Disc diffusion susceptibility testing of yeasts appears to be generally applicable. However, when results are equivocal quantitative test methods should be used.

Authors' summary

Combined activity of minocycline and amphotericin B *in vitro* against medically important yeasts

M. A. LEW, K. M. BECKETT AND M. J. LEVIN (1978). *Antimicrobial Agents and Chemotherapy*, 14, 465-469

The capacity of minocycline to enhance the activity of amphotericin B against *Candida albicans*, *Torulopsis glabrata*, *Cryptococcus neoformans*, and non-*albicans* *Candida* was examined *in vitro* using a time-killing curve technique.

Synergism was apparent at four hours with 5 of 5 strains of *C. albicans*, 8 of 8 strains of *C. neoformans*, and 1 of 12 strains of non-*albicans* *Candida*. Synergism was apparent at 24 hours with the remaining 11 strains of non-*albicans* *Candida* and all 5 strains of *T. glabrata*. *C. neoformans* was the most susceptible of the yeasts to the minocycline-amphotericin B combination; seven strains showed a 3-log or greater reduction in colony count in four hours and all strains showed this reduction in 24 hours at amphotericin B concentrations of 0.4 $\mu\text{g/ml}$ or less in the presence of minocycline.

Authors' summary

Prevalence of pathogenic yeasts and humoral antibodies to *Candida* in diabetic patients

F. C. ODDS, E. G. V. EVANS, M. A. R. TAYLOR, AND J. K. WALES (1978). *Journal of Clinical Pathology*, 31, 840-844

Recurrent vaginal *Candida* infection

(leading article) *British Medical Journal*, 1978, 2, 1735-1736

Genital herpes

Cellular immune response in genital herpes simplex virus infection

L. COREY, W. C. REEVES, AND K. K. HOLMES (1978). *New England Journal of Medicine*, 299, 986-991

We studied the relations between the cellular immune response, pre-existing complement-fixing antibody, and virus type with duration of virus excretion in genital herpes simplex virus (HSV) infection.

Thirty-six patients (seven with HSV-1 and 29 with HSV-2), with genital herpes underwent serological testing, sequential viral cultures, and weekly determination of lymphocyte-transformation stimulation index with inactivated HSV antigen. The duration of virus excretion was shortest in those with pre-existing complement-fixing antibody, was unrelated to virus type, and was inversely correlated with the magnitude of the mean peak stimulation index ($r=-0.69$, $P<0.001$). Prolonged virus excretion occurred in patients with a delayed and diminished peak index. Recurrent episodes had a higher peak index (29.4 compared to 14.5) ($P<0.02$), an earlier development of the peak during

recurrences (9.1 versus 25.8 days) ($P < 0.01$) and a briefer duration of viral shedding than initial episodes. Thus, the temporal course and magnitude of the stimulation index correlate with and may determine the duration of genital HSV infection.

Authors' summary

Sensitivity of the virus isolation and immunofluorescent staining methods in diagnosis of infections with herpes simplex virus

C. T. CHO AND K. K. FENG (1978). *Journal of Infectious Diseases*, 138, 536-540

Tissues from marmoset monkeys infected with herpes simplex virus (HSV, herpesvirus hominis) were used to evaluate the relative sensitivity and limitation of the virus isolation technique and the immunofluorescent (fluorescent antibody or FA) staining method for diagnosis of HSV infection. HSV encephalitis or disseminated infection or both in marmosets were established by intracerebral, intramuscular, or intravenous inoculation of the virus. Brain tissues, liver, spleen, kidney, adrenal gland, lymph node, and lung were harvested and prepared for the virus isolation procedure in tissue culture and for direct FA staining. Data from six marmosets infected with HSV type 1 and two infected with type 2 indicated that the virus isolation method was more sensitive and reliable than the FA staining technique. False-negative results by FA staining were found in two situations: (1) presence of focal lesions that were missed by the frozen sections; and (2) presence of low concentrations of virus in tissues ($\leq 3.5 \log_{10}$ 50% tissue culture infective doses/g). FA staining provides a rapid method for detection of viral antigens, but isolation of virus in tissue culture is required for a conclusive diagnosis of active infection.

Authors' summary

Herpesvirus type 2: study of semen in male subjects with recurrent infections

F. A. DETURE, D. M. DRYLIE, H. E. KAUFMAN, AND Y. M. CENTIFANTO (1978). *Journal of Urology*, 120, 449-451

Semen from 30 healthy male subjects with recurrent infections with herpesvirus type 2 was obtained when subjects were free of lesions and surveyed by tissue culture for an infectious virus in an attempt to elucidate the transmission of this disease. Inclusion bodies compatible with herpes-

virus were found in tissue cultures of semen from two participants but an infectious virus could not be cultured directly from any sample. The data suggest that herpesvirus type 2 is not ubiquitous in semen of male subjects with recurrent genital infections. The possible role of seminal inhibitors and a defective virus in causing the observed results is discussed, as are the current theories of herpesvirus type 2 transmission.

Authors' summary

Antibody-mediated recovery from subcutaneous herpes simplex virus type 2 infection

J. E. OAKES AND H. ROSEMOND-HORNBEAK (1978). *Infection and Immunity*, 21, 489-663

Proctitis associated with herpesvirus hominis type 2 infection

J. P. CURRY, J. A. EMBIL, C. N. WILLIAMS, AND F. R. MANUEL (1978). *Canadian Medical Association Journal*, 119, 485-487

Surgical treatment for recurrent herpes simplex

W. B. SHELLEY (1978). *Lancet*, 2, 1021-1022

Other sexually transmitted diseases

Genital warts: incidence of associated genital infections

G. R. KINGHORN (1978). *British Journal of Dermatology*, 99, 405-409

Two hundred and seventy-eight men and 200 women who presented to a special treatment clinic with genital warts were screened for accompanying genital infections. One hundred and twenty-nine (61%) of 212 presentations in women compared to 98 (32%) of 303 presentations in men were accompanied by another genital infection ($P < 0.001$). Recurrent presentation with warts was commoner in men ($P < 0.001$). In both sexes recurrences were less likely to be accompanied by another genital infection ($P < 0.002$).

Yeasts (25%), *Corynebacterium vaginale* (21%), *Neisseria gonorrhoeae* (12%), and *Trichomonas vaginalis* (12%) were the commonest pathogens in women. Non-specific genital infection (17%) and

gonorrhoea (10%) were the commonest accompanying infections in men.

The identification and treatment of these infections, especially in women, is important for the rapid eradication of warts, for, by increasing genital moisture they can create and maintain a favourable environment for wart proliferation. In addition to screening all patients with warts for other sexually transmitted diseases, women should be screened for yeasts and *C. vaginale*.

Authors' summary

Podophyllin poisoning. Systemic toxicity following cutaneous application

G. E. SLATER, B. H. RUMACK, AND R. G. PETERSON (1978).

Obstetrics and Gynecology, 52, 94-96

The toxicity of topically applied podophyllin in a 16-year-old girl is presented. Coma requiring respiratory support and major neurological complications as well as haematological and hepatic toxicity was observed. Therapy with a new modality, charcoal haemoperfusion, resulted in resolution of the acute toxicity, leaving a peripheral neuropathy, which had not completely resolved after four months. The pharmacology and suggested treatment measures for the toxicity of this rarely reported agent are reviewed.

Authors' summary

Condylomata acuminata in an infant and mother: report of a case

M. S. EFTAIHA, A. L. AMSHEL, AND

I. L. SCHONBERG (1978). *Diseases of the Colon and Rectum*, 21, 369-371

Miscellaneous

Genital occurrence of oral microbiota

U. FORSUM, E. HJELM, K. HOLMBERG, C.-E. NORD, AND J. WALLIN (1978).

Acta Dermatovenereologica (Stockholm), 58, 353-356

Recent studies indicate that tonsillar gonococcal infection or colonisation is fairly common. Carriage rates of about 8% have been found. These studies also indicate that oro-genital contacts are common. Since very little is known about the amount of oral microbiota transmitted to the genitals, we have studied

the occurrence of oral streptococci and *Neisseria* species in urethra and cervix. Among 128 patients attending an STD clinic we found 10 carriers of oral streptococci, one *Streptococcus mitis*, four *Streptococcus sanguis*, one *Streptococcus mutans*, and four *Streptococcus salivarius* and one case of urethritis due to *Neisseria meningitidis*. Seventy-three of the patients had recently had their genitals exposed to the oral flora of their partners. Despite the heavy contamination with oral

microbiota that can be assumed to occur in these cases, there seems to be no colonisation of the genitals with oral microbiota.

Authors' summary

Trimethoprim-sulfamethoxazole and minocycline hydrochloride in the treatment of culture-proved bacterial prostatitis

D. F. PAULSON AND R. D. WHITE (1978). *Journal of Urology*, 120, 184-185

Value of examining buffy coats for intragranulocytic micro-organisms in patients with fever

J-P. STUDER, M. P. GLAUSER, AND M. SCHAPIRA (1979). *British Medical Journal*, 1, 85

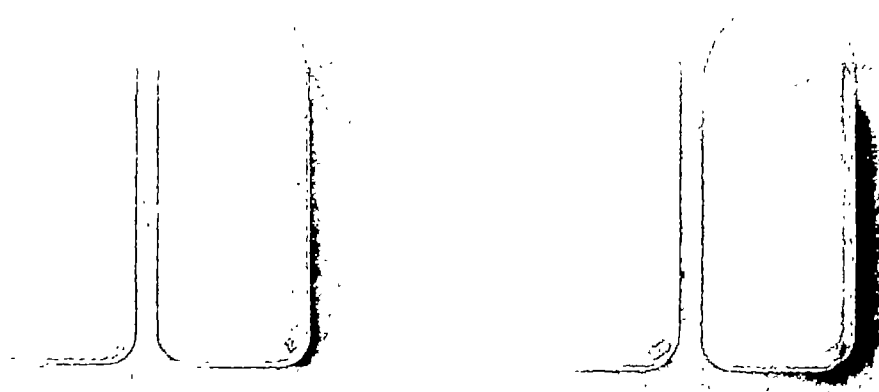
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Management of non-specific urethritis in men

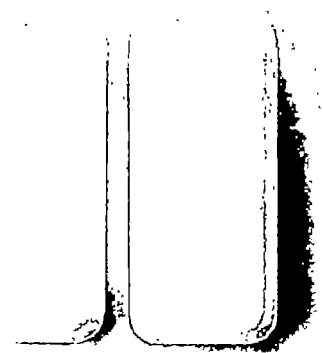
In the paper by O. P. Arya *et al.* (December, 1978, p. 415) the second sentence of the third paragraph should have read, '... ($\times 100$ objective $\times 5$ binocular eyepiece)'.

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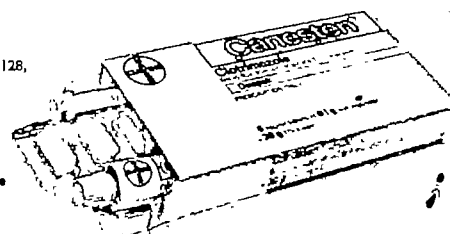
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A journal of the sexually transmitted diseases and allied disorders edited for the Medical Society for the Study of Venereal Diseases

Contents

Bulletin of the International Union against the Venereal Diseases and Treponematoses: 29th General Assembly, Leeds, 21-25 May 1978 *page 75*

Theme 1: Recent advances in the microbiology and immunology of gonorrhoea

Sensitivity of *Neisseria gonorrhoeae* to penicillin in West Germany D. PETZOLDT, K. GRUNDER, AND U. NEUBERT (West Germany) *page 80*

Effect of different preparation procedures on the ultrastructure of gonococci W. GEBHART, W. JURECKA, J. SÖLTZ-SZÖTS, J. THURNER, A. ELLINGER, AND K. KITZ (Austria) *page 83*

Preservation of *Neisseria gonorrhoeae* by the gelatin-disc method S. YAMAI, Y. OBARA, T. NIKKAWA, Y. SHIMODA, AND Y. MIYAMOTO (Japan) *page 90*

Theme 2: Recent advances in the immunology of syphilis

Role of humoral versus cellular mechanisms of resistance in the pathogenesis of syphilis M. METZGER (Poland) *page 94*

Attenuated yaws in Surinam P. L. A. NIEMEL, E. A. BRUNINGS, AND H. E. MENKE (Surinam) *page 99*

Comparison of cardiolipin and treponemal tests in the serodiagnosis of yaws H. E. MENKE, J. VELDKAMP, E. A. BRUNINGS, P. L. A. NIEMEL, A. NOTOWICZ, AND E. STOLZ (The Netherlands) *page 102*

Double-staining procedure for the FTA-ABS test E. F. HUNTER, R. M. MCKINNEY, S. E. MADDISON, AND D. D. CRUCE (USA) *page 105*

A note on microscopy F. J. AUMONIER (Gt Britain) *page 109*

Therapeutic effect of oral doxycycline on syphilis Y. ONODA (Japan) *page 110*

Theme 3: Recent advances in the study of STDs due to viral and other agents

Follow up of patients with hepatitis B surface antigen P. D. SIMMONS (Gt Britain) *page 116*

Group B streptococcal infection J. M. KNOX (USA) *page 118*

Vaccination for herpes simplex genitalis TH. NASEMANN AND S. W. WASSILEW (West Germany) *page 121*

Therapeutic aspects of herpes genitalis J. SÖLTZ-SZÖTS AND D. FANTA (Austria) *page 123*

Theme 4: New trends in the epidemiology of STDs

Illness behaviour and STDs R. M. HARRISON (Gt Britain) *page 125*

Critical evaluation of the venereal disease control campaign in Poland (1970-76) J. TOWPIK (Poland) *page 127*

Epidemiology of gonorrhoea and syphilis in South Australia (1966-77) D. JORGENSEN (Australia) *page 131*

Epidemiology of gonorrhoea and syphilis in New Zealand W. M. PLATTS (New Zealand) *page 138*

Does gonorrhoea surveillance pay? R. C. RENDTORFF AND H. PACKER (USA) *page 142*

An alternative to the problem of insufficient physician manpower in the clinical care of STDs C. W. PARTON AND S. M. SGROI (USA) *page 144*

Results of routine screening for STDs in Austria over a six-year period D. FANTA, W. GEBHART, W. GROSS, E. M. KOKOSCHKA, J. SÖLTZ-SZÖTS, AND J. THURNER (Austria) *page 147*

Epidemiological importance of concealed nongonococcal urethritis R. R. WILLCOX (Gt Britain) *page 149*

Abstracts *page 154*

Notice *page 159*

Correction *page 159*

June 1979 Vol. 55 No. 3

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¹Thin RN, Shaw EJ. Diagnosis of gonorrhoea in women.

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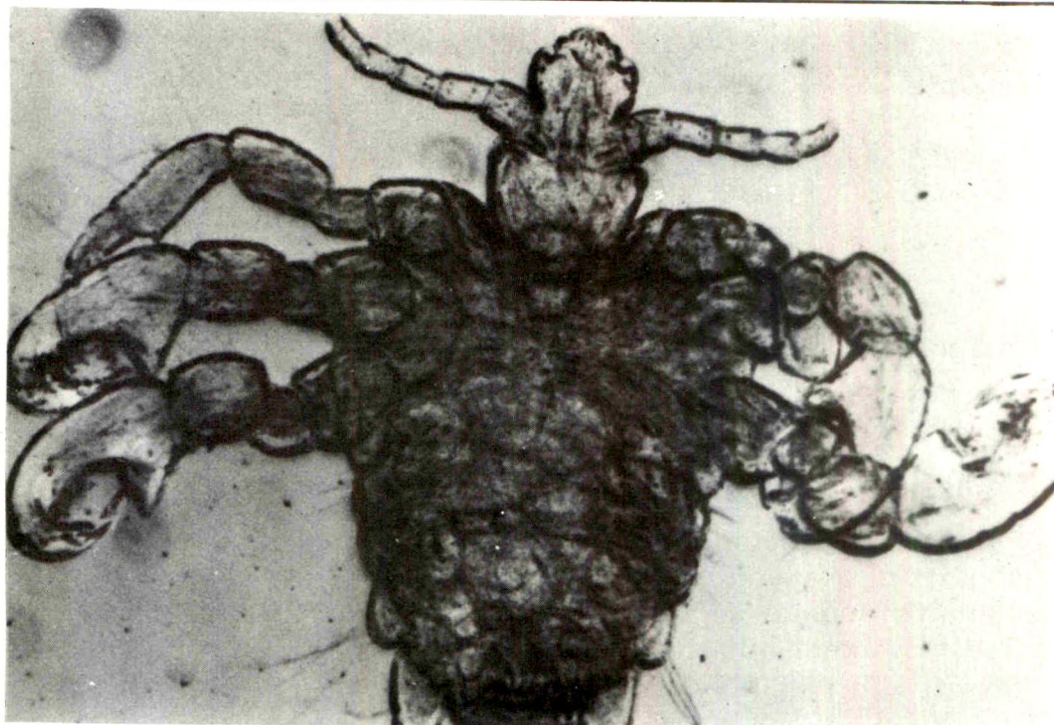
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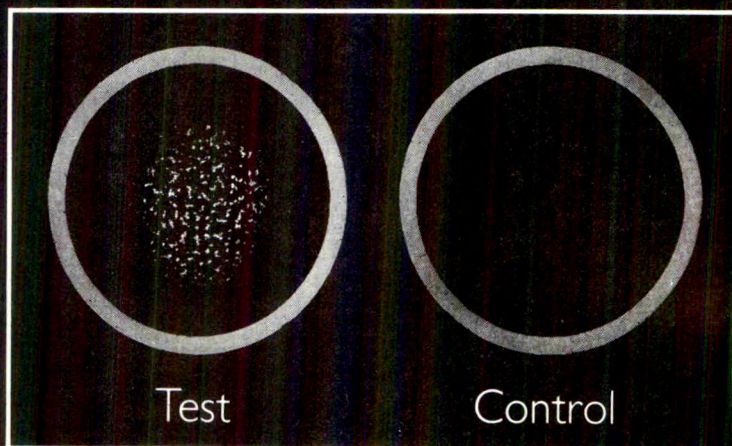
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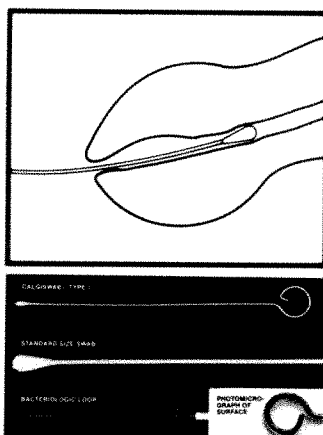
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The Harrison Lecture, 1978

Sexually transmitted diseases and travel

Passage to India, 1971

C. S. NICOL

From St Thomas's Hospital, London

I feel greatly honoured to have been asked to give the third Harrison Lecture, following such giants in the subject as Ambrose King and Tommy Turner. It is appropriate, however, that it should be given at St Thomas's Hospital—Colonel Harrison's old hospital. I am taking as my subject the importance to the individual of travel abroad in the study of sexually transmitted diseases (STDs) and, in particular, the planning and execution of a visit to India in 1971 by invitation of the British Council.

I am sure that Colonel Harrison would have been the first to agree on the value of experience gained in travel, whether obligatory, as in the armed Forces in two world wars, or, voluntarily, as in World Health Organisation (WHO) fellowships or in attendance at meetings of the International Union for the Venereal Diseases and Treponematoses (IUVDT) and the Medical Society for the Study of Venereal Diseases (MSSVD). It is no coincidence that a photograph of Colonel and Mrs Harrison attending an international meeting appears in the first Harrison Lecture (King, 1974). It is most important to take one's wife to such meetings whenever possible.

In the second world war, I was fortunate enough to be a member of Ambrose King's team between 1941 and 1945, firstly at the Royal Victoria Hospital, Netley, and later at the Military Hospital, Westbury. The hyperthermia unit, in which I was one of the first guinea pigs, was established as a result of Ambrose King's pre-war visit to the United States. This visit also fostered his close friendship with Dr Earle Moore, which continued during and after the war and resulted in the opportunity for a series of year-long fellowships for young physicians at the Johns Hopkins Hospital (Medicine I Department) under the inspired guidance of Dr Moore. I was very

fortunate to be the first to be nominated by Ambrose King in 1949.

For many years meetings of the IUVDT have been held in various host countries twice every five years, one of them to coincide with the international meeting of dermatologists. The programme of these meetings has mainly concerned the social aspects of STD. My first experience of such a meeting was at Naples in 1955, as a representative of the BFVD, which Colonel Harrison helped to form with a very generous donation.

Meetings of the MSSVD used to be held only in London, but more recently a meeting has been held every alternate year in the provinces. In 1961, when I was secretary of the society, a meeting was held in Paris with the co-operation and enthusiasm of Pierre Durel and André Siboulet, and I am glad to say that meetings abroad every alternate year are now the regular policy of the society.

Fellowships ranging from a few months to a year or more were given by the WHO in the post-war era, and meetings of experts in Geneva were arranged at regular intervals. Many young doctors remember with gratitude the help and advice of Thorstein Guthe. Dick Willcox has worked closely with the WHO and has a world-wide reputation.

In recent years hospital and regional funds available for travel in the study of STDs have diminished, and I am glad to say that this fact has been appreciated at the Department of Health and Social Security (DHSS). As a result funds have been provided during the last few years for travel abroad for doctors, nurses, and ancillary staff working in the field of STD.

Introduction to India

It is to all those who plan to take advantage of such facilities in the future that I give some details of the planning of a travel project I was invited to undertake in 1971. In the spring of that year the

Address for reprints: Dr C. S. Nicol, Lydia Department of Genitourinary Medicine, St Thomas's Hospital, London SE1 7EH

Received for publication 15 August 1978

British Council invited me to plan with them a visit by air to India to report on the situation regarding STDs. The visit was to start in late October and to last four weeks. It is worth remembering that air fares can cover stops en route, and it was agreed at an early stage in the planning that an extra two weeks should be spent on a visit to Afghanistan on the outward journey and a visit to Iran and the Lebanon on the return journey. I will confine myself to the preparation of the programme for the four weeks in India, visiting New Delhi, Calcutta, Madras and Vallore, and Bombay and Poona. It was my first visit to the sub-continent (Fig. 1).

In *The Times* newspaper of 13 January, 1978, Bernard Levin commented: 'I am off to India for the first time. How many educated Englishmen know anything of Indian history earlier than when the British and French came? How many Europeans even know that the Greeks were there two millenia

earlier? What do we know of the Moghul Empire or of the strange migration of Buddhism from the land of its birth? Precious little, most of us.' This state of affairs can be put to rights by reading the necessary guides and reference books on the geography and history of the sub-continent and thus gaining some knowledge of its art and literature, its sociology and current affairs.

PREPARATION

At least three prepared lectures on STD with slides should be taken for a trip of this duration. A working, five-day week was agreed, which allowed me to see places of historical interest. A tour abroad can be ruined by illness, so the DHSS handbook (DHSS, 1977) should be carefully studied. The risk of contracting malaria or infective hepatitis should not be underestimated; besides routine vaccination, as well as cholera and TAB inoculations, it is also

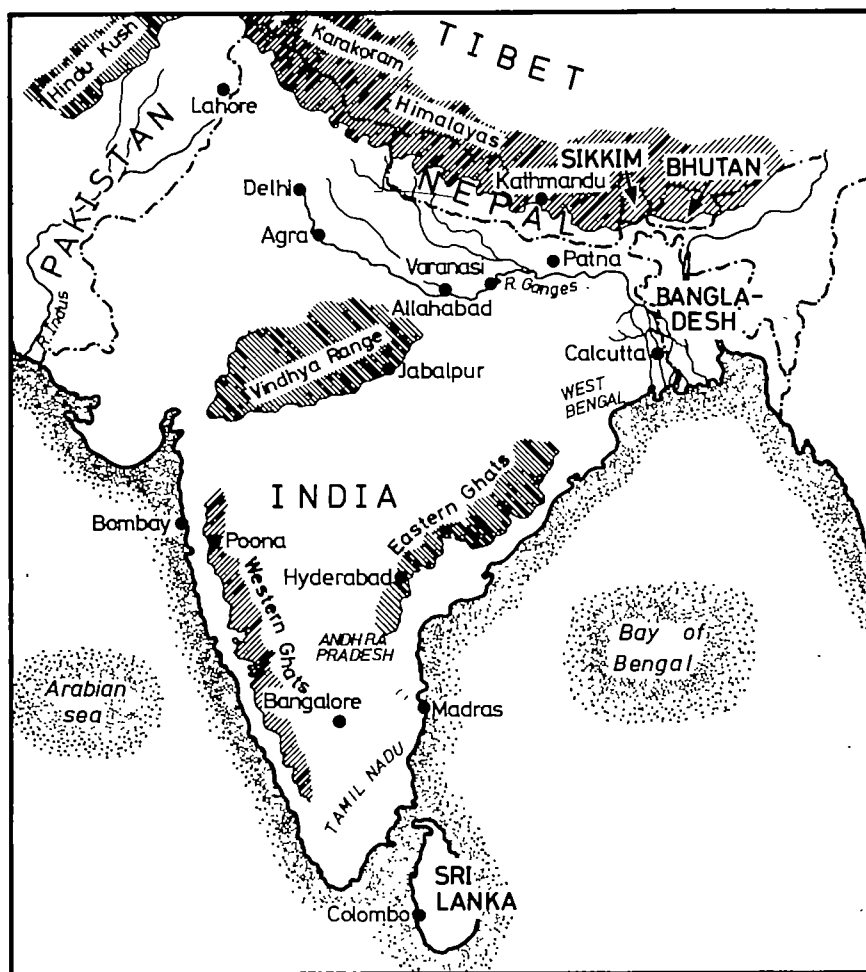


Fig. 1 Map of India

easy to protect against polio. Symptomatic and antibiotic treatment for gastroenteritis should be carried. Anti-sunburn lotions and insect repellents may be useful; even the STDs are mentioned as a hazard. Remember it is pleasant to be able to acknowledge hospitality given on a tour by taking your hostess a small present.

GEOGRAPHY

From east to west in the northern regions lie the Himalayas, the Karakoram, and the Hindu Kush mountains (now in Pakistan), which supply the watershed for the two great river systems, the Ganges and the Indus (also now in Pakistan). To the south of these lie the Vindhya Range, the Deccan plateau, the Eastern and Western Ghats, and southern India. The historic invasion routes were through the Karakoram, Khyber and Bolan passes, and by sea via the Bay of Bengal and the Arabian Sea. The plains of India can be visited between October and April but become very hot in May and June until the south-west monsoon rains come in July, August, and September; the north-east monsoon brings rain to the south-eastern parts of India from December to February.

HISTORY AND RELIGION

The history of India starts with the civilisation of the Indus valley—dating back to 2500 BC—where excavations have taken place at the main sites of Harappa and Mohenjo Daro (now in Pakistan), which has a population of 20 000; there was advanced town planning in a priest-dominated society.

Aryan invasions from the north occurred in 1500-500 BC; these people from central Asia were nomadic and were ruled by kings (Rajas). They conquered and absorbed the Indus civilisation and drove the more primitive and dark-skinned Dravidians into southern India. The Aryan language was Sanskrit and 'vedas' (sacred Hindu hymns) were composed at this time. The caste system of (a) the Brahmins (priests, teachers, and scholars), (b) the Kshatriyas (warriors and administrators), (c) the Vaisyas (the mass of the people, including traders and artists), and (d) the Sudras (serfs, the old untouchables) evolved. Hindus believed that pollution occurred if there was contact with a member of a lower caste.

Hinduism

Hinduism is a blend of countless cults and gods developing into a vast mythology dominated by three gods: Brahma (the creator); Siva (the destroyer); and Vishnu (the preserver). Vishnu has 10 incarnations (avatars) and, of these, two—Rama and

Krishna—are the most important. Rama was the hero of the epic, *Ramayana*, and Krishna was the hero of *Mana Bhanta*. The gods had consorts, such as Parvati, the consort of Siva—the most powerful goddess—who could also appear in an aggressive form as Kali or Durga. Animals venerated by the earliest Indian societies were joined to the Hindu pantheon as companions of major deities.

Some useful terms in Hinduism are:

Karma—ethical cause and effect, the natural law, the belief in reincarnation

Dharma—duty, moral code

Bhakti—devotion to a personal god (such as Krishna)

Yoga—physical, mental, and psychic techniques in body control

Tantra—erotic mysticism

Buddhism

Around 570 BC a Hindu prince, Siddhartha (Gautama Buddha, 'The Enlightened One'), was born in northern India; he is considered to be another avatar of Vishnu. Two types of Buddhism developed: (a) the Lesser Vehicle or Hinayana, with Buddha represented as a teacher who reaches Nirvana, the state when no further reincarnation is needed; and (b) the Greater Vehicle or Mahayana, with Buddha as a god and many Bodhisattvas, who reject ultimate Nirvana in order to help humans on earth.

Buddha in his lifetime preached four Noble Truths: (a) life is suffering; (b) the reason for suffering is craving for individual fulfillment; (c) the duty is to overcome this; and (d) the means is the eightfold path of right understanding, right purpose, right speech, right behaviour, right means of livelihood, right effort, right awareness, and right meditation. Later in the Greater Vehicle it was recognised that Buddha was a god and that heaven and hell existed.

Islam

A comparatively new religion from Arabia was brought to India by Arab invaders in the eighth century AD. Moslems devoted their lives to Islam or to submission to God. The religion of Islam preached a rigid monotheism—that is, that there is a God and Mohammed was his prophet. Finally, Islam held that all Moslems were brothers and that all men were equal before God, regardless of their class or colour; their Bible is the Koran. The Sunni sect are in the majority in India; the Shia sect, with different laws and stricter rules, are in the majority in Afghanistan and Iran.

Mauryan Empire

There were three great Indian empires before the

Europeans appeared on the scene. In 327 BC Alexander the Great, having vanquished the Persians, invaded India and met and defeated King Porus on the Indus; however, two years later his army forced him to retreat. In this power vacuum the Mauryan kings conquered all India by 300 BC. Their great emperor, Ashoka, adopted Buddhism as the state religion. He also set up rock and pillar edicts all over his domain.

Gupta Empire

Between AD 400 and 500 a new power, the Guptas, controlled all northern India. Buddhism was on the wane and had lost popular support, while the Indian epic, the Hindu *Ramayana*, was composed, and modern, orthodox Hinduism was established. The Guptas were overthrown by Huns from central Asia. These were followed by Moslem, Arab, and Turk invasions in the eighth and twelfth centuries and were opposed in northern India by the Hindu Rajputs. Delhi Sultanate was founded in the twelfth century but it was sacked by Timur at the end of the fourteenth century. By this time the Pahlava Empire in southern India held sway from the fourth to ninth centuries and was at its height in the seventh century AD.

Moghul Empire

A new wave of Islamic invasions was led by Babur, a descendant of Ghengis Khan and Timur, after he was driven from Samarkand. He captured Agra and Delhi in AD 1526 and founded the Moghul Empire. He was succeeded by his son, Humayun, who promptly lost control of the empire and was in exile for 15 years. He returned to Delhi in 1556, however, but died the next year and was succeeded by his son, Akbar, the greatest of the Moghuls (a contemporary of Elizabeth I of England). He founded a new monotheistic religion (Din Illahi) in 1582 and married a Hindu princess. His son, Jahangir, was weak and frivolous; his Persian wife, Nur Jahan, held the power. He was succeeded by Shah Jahan, who built the Taj Mahal in memory of his wife, Mumtaz Mahal. He was deposed and imprisoned by his son, Aurangzeb, an Islamic fanatic who persecuted the Hindus and weakened the empire; he died in AD 1707 after subjugating southern India. Since the arrival of the Portuguese in Goa in 1525, and the founding of the East India Company in the seventeenth century, the scene was being set for the establishment of the British Raj. It remained for Lord Curzon, when viceroy, to restore Indian architectural gems and re-establish interest in the three great Indian empires I have described.

CONTEMPORARY HISTORY

India, when we visited it in 1971, was the largest

democracy in the world, with a population of over 550 million and territories of over 1¼ million square miles. Its principal language was Hindi, spoken in southern India, but altogether there were over 800 other dialects. There were 60 million Moslems and another 30 million non-Hindus. The numerous sects which had split from Hinduism included the Sikhs and Jains; Buddhism, as an active religion, had by that time disappeared from India. The main population was rural with 80% employed on the land and only 20% living in the urban areas. Tribes occupied some of the forest areas, and they had no caste system.

India became an independent State in August 1947. It was a secular State dedicated, under the influence of Gandhi and his Congress Party, to the gradual elimination of the more archaic anomalies of Hindu law and custom—such as the caste system—to relieve the plight of the untouchables. Sati (suttee), the burning of widows, had been made illegal under the British Raj. Equal rights were given to all racial and religious minorities, and a federal system divided power between the central government and the various states, but the central government had the right to take over power if state administration broke down. The Government of India with Mrs Indira Gandhi, as premier, represented the left wing of the split Congress Party; she gained a large majority at the polls in the mid-term election. The troubles in East Pakistan resulted in millions of refugees fleeing into West Bengal and precipitated a crisis with Pakistan which brought the country to the brink of war. India, as a developing country, was the largest receiver of foreign aid, some £466 million a year. The income of the average Indian, however, was still only £30 a year.

To bring us up to date, in 1971 the India-Pakistan conflict broke out and resulted in the defeat of Pakistan and the emergence of independent Bangladesh. Then followed, in 1975, the emergency rule of Mrs Indira Gandhi and finally her defeat at the polls by Mr Morarji Desai and his Janta party. Now there are actions in the courts against both Mrs Gandhi and her son, Sanjay; there is evidence which suggests that male sterilisation to control a population explosion was not always voluntary.

MEDICAL MANPOWER

In 1966 there were nearly 200 000 doctors registered in India with 2000 (1%) in the United Kingdom and 2000 (1%) elsewhere abroad. There were 85 medical schools (short of many hundreds of teachers). Now, 15 000 Indian doctors in the United Kingdom are members of the Indian Medical Association. The ancient Indian medical term, 'ayurveda', means that men should use health as a means of developing

higher pursuits; most doctors in India, however, follow the Western tradition nowadays.

SOCIOLOGICAL ASPECTS

To quote from *Which Country?* by Stark Murray (1967): 'It is very difficult to find out what young people think about love, sex, and marriage in India, for they have not yet reached the stage of public discussion on such matters.'

Arranged marriages suggest a separation of emotional love from sexual intercourse, and older Indian doctors sometimes argued that this was a better state of affairs than the Western concept of the one as part of the other. From this it would appear that India has no need to worry about the problems, which occupy so much time and effort in Christian countries, such as premarital sexual intercourse, chastity, and sexual morals in general. But there seems to be a ready acceptance of prostitution—indeed some advocacy of its essential place in society; and quite definitely there is considerable masculine belief still in two different sexual codes. Between 1965 and 1970, 5½ million Indians were sterilised, and in 1969 alone 53 million contraceptives were distributed under the family planning scheme.

A most detailed study of Indian culture and society is given in the book, *The Speaking Tree*, by R. Lannoy (1971). It attempts to identify the sources of the difficulties which India is currently experiencing; to quote: 'There is probably nothing in the life of foreign peoples more prone to misconception than the way they conduct their sexual life. India is no exception; the foreigner often forms a coloured, not to say lurid, picture compounded of erotic temple sculpture, the Kama Sutra, mystical orgiasticism, and Bombay's "street of the cages"'. At the same time, while he has probably heard about the cult of phallic deities (lingam), the foreigner also notices a counter-emphasis in religion on asceticism, a puritanical social life with a tabu on overt expression of tenderness between young people, and sentimental movie romances in which kissing is prohibited. All these things exist, of course, while the Indian cultural heritage projected by the tourist industry is also complex, but if we are to understand it we must penetrate the screen of romanticism to the lives of real men and women. It seems that every alternate generation in the West produces the fantasy of a heightened Indian sexuality; the cult of psychedelic Hindu eroticism in the 1960s was just such a case. This too we must circumvent if anything like an objective assessment is to be made.'

Two articles by Peggy Holroyde (1971), a social worker, reviewed the revolt of youth in the West and concluded that Indian urban civilisation seemed 'hell

bent' on imitating the West, although she saw some hope in the 'psychological safety valve' of Hindu philosophy. *The Twice Born* by Carstairs (1971) gave details of promiscuity in village life and a high incidence of venereal disease in some tribes.

Tour

NEW DELHI

On our arrival in New Delhi on 8 October, the British Council was most helpful in arranging hospital, laboratory, and research centre visits. Outstanding among these visits was that to the All-India Institute of Medical Sciences, under the direction of Professor Bhutani. Visits were also made to the Department of Health and the regional office of the WHO. The British Council also supplied hospitality for me to meet key hospital personnel. A medical conference was attended and two lectures were given. Finally, a press conference and an interview on All-India Radio were arranged. Adequate time was available for sightseeing in Delhi and Agra (Fig. 2), where a further lecture was given.



Fig. 2 *Taj Mahal, Agra*

CALCUTTA

From Delhi we flew to Varanasi and then on to Kathmandu, where we got a brief view of Mount Everest. We finally arrived in Calcutta on 19 October. The British Council arranged a visit to three hospital clinics and a laboratory. I met the director of health services and gave two lectures. We visited the National and Victoria Museums and were taken round Flagstaff House.

MADRAS

We arrived in Madras on 24 October. A British Council car drove us direct to Vallore. We stayed in the compound of the Christian Medical College founded by an American missionary, Dr Ida Scudder, in 1902. The hospital in Vallore now has

1200 beds and a private wing. A lecture was given there. On our return to Madras we visited the Poonamallee Health Unit, which was receiving financial support from the Rockefeller Foundation. There were eight sub-centres covering a population of 90 000 in 39 villages. On my return to Madras I met the State Minister of Health.

My main visit in Madras was to the Institute of Venereology. We were met by Dr Rangiah, the ex-director, in the absence of the director, Dr Sowmini. We inspected the clinic, seeing both the male and female sections in action, and met members of the staff. Inpatient cases were discussed on a ward round. Many patients with congenital and cardiovascular syphilis and neurosyphilis were seen, which are now rare in the United Kingdom. Diagnosis, prognosis, and treatment were discussed in a 'grand round'. A lecture was given, which was well attended. The next morning, recent advances in the diagnosis of syphilis and gonorrhoea and in the aetiology of non-specific genital infection were discussed at a well-attended clinic staff gathering. A campaign to combine the control of venereal diseases with family planning was described. We also visited the Venereal Disease Reference Laboratory with Dr Chacko, the ex-director, in the absence of the director, Dr Krishnan. New laboratory techniques were discussed and the various research projects in progress in the laboratory were inspected. The laboratory's syphilis serology proficiency testing programme was demonstrated; in 1969 and 1970 participants included 49 other laboratories from all over India. We enjoyed two trips to see some of the southern Indian temples and monuments (Fig. 3).



Fig. 3 Mahabalipuram, Southern India

BOMBAY

We arrived in Bombay on 1 November. This city appeared to be the most prosperous and modern in India, although of course there were some slum areas. The British Council arranged a visit to five

hospitals, of which the Grant Medical College was the most impressive. I was shown round by Dr Desai, and two lectures were given.

We also visited Poona, staying at the Turf Club (memories of the Raj). At the Armed Forces Medical College we met General Hoon, the commandant, and then visited Hindustan Antibiotics. Their library was claimed to be the best stocked in India, and included the *Russian Journal of Venereology*; I suggested the *British Journal of Venereal Diseases* would be a useful addition.

An extra visit by air to Aurangabad was arranged at the last moment; the hospital was visited and a lecture given. Aurangabad is the regional centre of Maharashtra State, where there is a high proportion of Moslems. We were able to visit the famous Ajanta and Ellora caves and temples by car and returned by air to Bombay, which ended the Indian part of our tour.

Conclusions and comments

The main objective of the visit to India was to separate the subjects of venereology and dermatology as far as possible and to establish centres in the main cities, including an up-to-date laboratory service with at least one reference laboratory. All this would only be possible if there was a satisfactory career structure in venereology up to the level of professor.

Adequate teaching of STDs at both undergraduate and postgraduate levels must be more organised. Indian doctors and administrators showed unlimited goodwill and enthusiasm to improve standards in the control of STDs (with the exception of a few hard-line dermatologists). The United Kingdom has, in general, better training facilities to offer in this field than either the United States or the USSR, as confirmed by Professor Turner (1976).

There are few reliable statistics on the incidence of the STDs—or even of the trends for the two main diseases, syphilis and gonorrhoea—but what evidence there is suggests that the incidence might be a hundredfold higher than that in the United Kingdom.

The Institute of Venereology and the Reference Laboratory in Madras set the standard that the other main urban centres should follow. The Grant Medical College in Bombay and the combined All-India Institute of Medical Sciences, and the Safdarjang Hospital in Delhi, also come near these standards. The situation in Calcutta is more difficult, but if the university created a chair in venereology it would have good support from the Venereal Disease Reference Laboratory.

In the district venereal disease centres the situation

was indeed desperate. They had small, overcrowded premises, inadequate staff, and in some cases not even the necessary curative drugs. These factors tended to force those who could afford to pay any fee, however small, into the hands of the private medical sector—or even of ‘quacks’—or into buying drugs direct from the chemist’s shop. Despite the federal government’s plans to support these clinics, they would never be operative unless the main urban centres improved and expanded so that they could train enough postgraduates to man the district clinics; only the state of Tamil Nadu seemed to be making any effort in this direction.

Until an immense effort is made to improve the various methods of contact-tracing, complete control of syphilis or gonorrhoea will not be possible and the reservoir of symptomless women will remain. After all, we have not yet got gonorrhoea under control in the United Kingdom, despite a highly sophisticated contact-tracing system.

Health education of the public should be promoted through a scheme similar to that used in teaching family planning. The close-knit structure of the Hindu family was beginning to break down and children no longer accept that their parents had the right to arrange their lives. There was also evidence that the young people in the cities were becoming more permissive. It would be possible to educate these young people by showing slides or short films at cinemas—which are always crowded—and by the other mass media as they develop. There was a need to monitor the trends in permissive attitudes in young Indian girls. In the student class the girl who was engaged might have premarital sexual intercourse, which would lead to further sexual experience if the engagement was terminated and so on thus leading to promiscuity. In the United Kingdom, at present only about 10% of gonococcal infections are acquired from prostitutes, while in India—although the figure may now be 90%—it is highly likely that it will decrease, as young men realise they can get their sexual experience, together with some emotional relationship, free with an amateur.

There was a great need to support those centres which set a high standard in venereology. Further help could be given by the British Council and other organisations by supporting requests for short-term visits to the United Kingdom by clinicians, laboratory workers, and contact-tracers working in the field of STD.

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Editor’s note

THE POSITION IN 1978

The specialty of venereology (sexually transmitted diseases, STD) is still practised along with dermatology and leprosy as a combined specialty in most parts of India, except in Tamil Nadu, Andhra Pradesh, and West Bengal, where all these subspecialties are independent. There is an adviser in STD to the Government of India; the post goes to the most senior dermatovenereologist in the central general government cadre, posted to Delhi.

There are two main associations, the Indian Association of Dermatologists, Venereologists, and Leprologists (a combined association) and the recently formed Indian Association for the Study of Sexually Transmitted Diseases.

The subject of venereology (STD) is taught at the undergraduate level by the dermatovenereologist. At the postgraduate level, dermatovenereology is a combined specialty, except in Tamil Nadu where Madras University awards a specialist postgraduate MD. Recently, the Indian Academy of Medical Sciences has allowed postgraduate candidates to opt either for dermatology or venereology as separate specialties or to take dermatovenereology as a combined specialty.

Ultrastructural study of satellite lymph nodes in syphilitic rabbits

RAMAH W. LAPUSHIN,* ROBERT E. BAUGHN, DANIEL M. MUSER, AND PHYLLIS GYORKEY

From the Departments of Medicine (Infectious Disease Section), Microbiology and Immunology, and Dermatology, Baylor College of Medicine, Houston, and the Veterans Administration Hospital, Houston, Texas, USA

SUMMARY In an electron microscopy study of lymph nodes from syphilitic rabbits plasmablasts and plasma cells were unequivocally identified in germinal centres. Up to 20% of the plasma cells possessed unusual cytological features. Paracortical hypoplasia was shown to be associated with actively phagocytic histiocytes. These findings may reflect morphological correlates of the aberrant immune regulation which has been observed in infection with *Treponema pallidum*.

Introduction

Lymphadenopathy is a characteristic of early syphilis. Light microscopy examination of syphilitic human lymph nodes has shown cortical hyperplasia with prominent germinal centres (Harstock *et al.*, 1970; Turner and Wright, 1972) and atrophy of paracortical (thymus-dependent) areas (Festenstein *et al.*, 1967; Levene *et al.*, 1969; Turner and Wright, 1972). Unusual findings have included histiocytic infiltration of the paracortex and the presence of what appear to be plasma cells in germinal centres; in normal lymph nodes or in other infections these cells are usually confined to medullary areas (Turner and Wright, 1972). Infection with *Treponema pallidum* is accompanied by alterations of immune regulation affecting antibody production and cell-mediated immunity (Wright and Grimble, 1974; Musher *et al.*, 1977); these include production of non-treponemal antibody (Turner and Hollander, 1957; Turner, 1970), suppression of the IgG response to a thymus-dependent antigen such as sheep red blood cells (Baughn and Musher, 1978), delayed production of lymphokines (Wicher and Wicher, 1977), and suppressed lymphocyte transformation to a variety of antigens and mitogens (Levene *et al.*, 1969; Musher *et al.*, 1974; Friedman and Turk, 1975;

Musher *et al.*, 1975, 1977; Pavia *et al.*, 1977). In the light of these abnormal immune responses we have undertaken an electron microscopy examination of lymph nodes from syphilitic rabbits. Our study unequivocally identified plasma cells in germinal centres and showed them to have several unusual morphological abnormalities. Ultrastructural examination also showed the paracortical histiocytes to be actively phagocytic. These abnormalities may be related to the changes in immune regulation which have been observed in syphilis.

Material and methods

RABBITS

Outbred New Zealand white male rabbits weighing 2-3 kg were housed individually in stainless-steel cages at an ambient temperature of about 18°C. Rabbits with a positive Venereal Disease Research Laboratory (VDRL) test reaction were excluded because of the possibility that this resulted from a subclinical infection with *Treponema cuniculi*.

ORGANISMS

T. pallidum (Nichols strain) was maintained by intratesticular passage in rabbits. Inflamed testes were removed aseptically, minced in sterile saline, and ground with sand with a mortar and pestle. After centrifugation at $270 \times g$ for 30 minutes to remove sand and cellular debris, the number of treponemes in the supernatant was determined by darkfield microscopy. Rabbits used in this study were shaved

*Dr LaPushin died on 19 January 1979

Address for reprints: Dr D. M. Musher, Infectious Diseases Section, Veterans Administration Hospital, 2002 Holcombe Boulevard, Houston, Texas 77211, USA

Received for publication 14 March 1978

on the back and infected intradermally with 10^7 organisms per site to a total of six to eight sites per rabbit.

OBTAINING, PROCESSING, AND EXAMINING LYMPH NODES

Lymph nodes are virtually never palpable in normal, uninfected rabbits. Early in the course of syphilis, however, satellite lymph nodes may be found draining the chancres in the fore and hind regions. These nodes were sampled at one, two, four, eight, 10, and 16 weeks after intradermal inoculation. At least two rabbits were studied for each time interval.

Rabbits were killed by an intravenous injection of air. Lymph nodes were excised rapidly and immediately placed in 1% phosphate-buffered glutaraldehyde or paraformaldehyde-glutaraldehyde (Karnovsky, 1965) and then in 1% phosphate-buffered osmium tetroxide; they were all stained together with uranyl acetate, dehydrated in a graded series of alcohols followed by propylene oxide, and embedded in Araldite-Epon (Araldite 506 and Epon 812, E. F. Fullham, Inc., Schnectady, N.Y. 12301, USA). Lymph nodes from these same rabbits were processed for conventional histology by fixing in Bouin's solution, embedding in paraffin, sectioning at $5\text{ }\mu\text{m}$, and staining with haematoxylin-eosin.

Sections were cut on a Porter Blum MT II ultramicrotome. Semi-thin ($1\text{ }\mu\text{m}$) sections were stained with methylene blue (Reynolds, 1963) and used for orientation. Thin sections were stained with uranyl acetate (Richardson *et al.*, 1960) then lead citrate (Reynolds, 1963) and examined with a Phillips 200 electron microscope.

Plastic $1\text{ }\mu\text{m}$ sections were studied for each time interval. To estimate the degree of cortical hyperplasia 500 cells from the centre of well-developed germinal centres were examined and the percentage of cells which contained mitotic figures was calculated (Table). For each time interval observations on five slides from five different samples were averaged.

Results

LYMPH NODE CORTX

Light microscopy examination of $1\text{ }\mu\text{m}$ sections showed cortical hyperplasia with prominent germinal centres; lymphoblasts predominated, appearing as large cells with centrally placed nuclei, well-defined nucleoli, and extensive euchromatin. The cytoplasm contained many polyribosomes, rough endoplasmic reticulum, and mitochondria (Fig. 1). Cells ranging in maturational stages from large to small lymphocytes constituted the remainder of lymphoid

Table Results of sampling of satellite lymph nodes from syphilitic rabbits

Week	Specimen no.*	% Mitotic cells†
1	117	4.0
	139	4.5
	176	3.2
2	124	2.9
	145	2.6
	182	2.7
4	127	0.8
	163	0.5
8	145	1.7
10	146	4.3
16	152	3.9

*Each number represents a different rabbit lymph node; these were minced and five samples were studied from each specimen.

†Each value represents the average of mitoses in five samples.

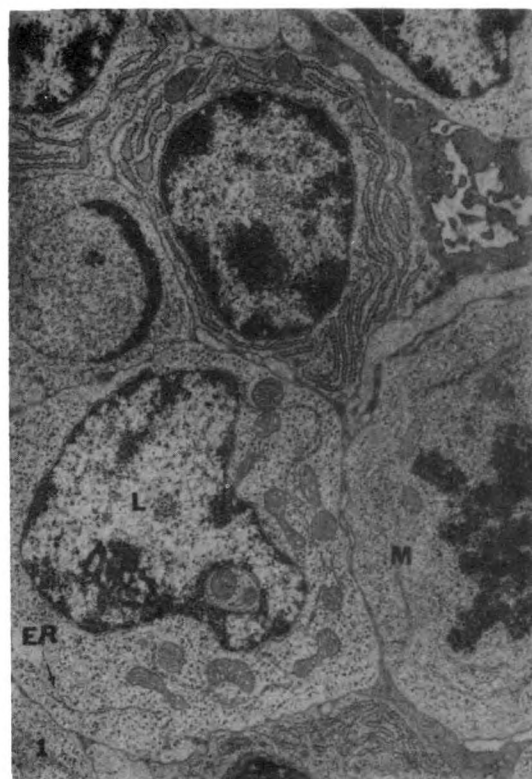


Fig. 1 An electron micrograph showing a typical area from a germinal centre of a syphilitic lymph node draining a seven-day chancre. Note the mitotic cell (M), a lymphoblast (L) with modest endoplasmic reticulum (ER) and mitochondria, and a plasmablast (Pb) with dilated endoplasmic reticulum (magnification $\times 7000$).

cells that were present. A striking finding was the presence of plasmablasts and plasma cells in germinal centres (Figs. 1 and 2), these cells usually being found

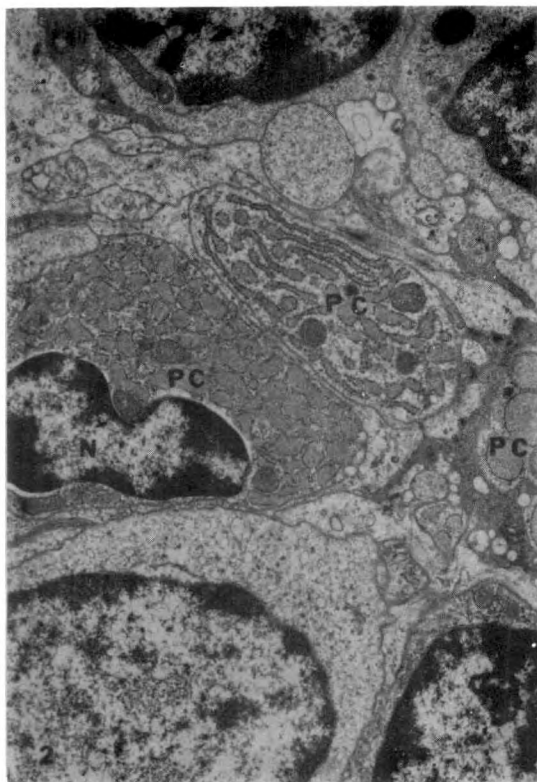


Fig. 2 An electron micrograph showing a characteristic plasma cell (PC) in a germinal centre. The cell is identified by its eccentric nucleus (N) with extensive margined heterochromatin and abundant well-developed dilated endoplasmic reticulum. Sections of cytoplasm of two adjacent plasma cells can be noted (magnification $\times 7200$).

only in the medulla. Typical plasma cells contained eccentric nuclei with heterochromatin dispersed along the periphery. They had large amounts of dilated rough endoplasmic reticulum and the Golgi apparatus was often prominent. Up to 20% of plasma cells had cytological abnormalities, including polymorphic nuclei, multiple nucleoli or morphological alterations of the Golgi apparatus or both (Figs. 3 and 4). Immature plasma cells, the plasmablasts, had moderate amounts of endoplasmic reticulum, centrally placed nuclei, and some heterochromatin. Hyperplasia of germinal centres, as determined by the percentage of mitotic cells, was prominent in the first two weeks, declined by the fourth week, but again was present at the tenth and sixteenth week (Table).

LYMPH NODE PARACORTX

Light microscopy examination of 1 μm sections

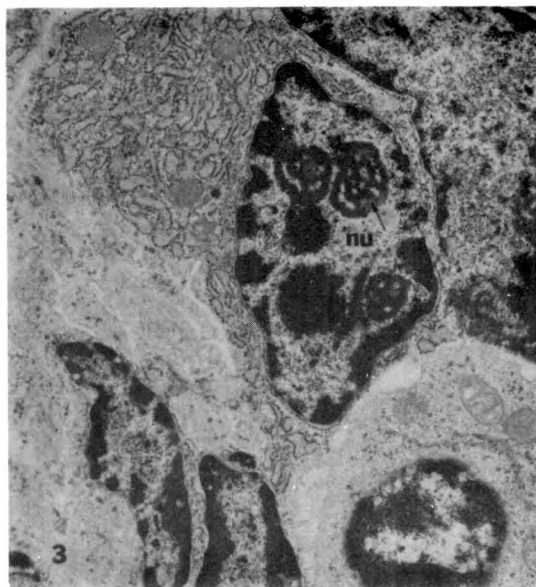


Fig. 3 The ultrastructure of an anomalous plasma cell. Note three distinct nucleoli (nu) in a cell with well-developed cytoplasmic endoplasmic reticulum (magnification $\times 4300$).

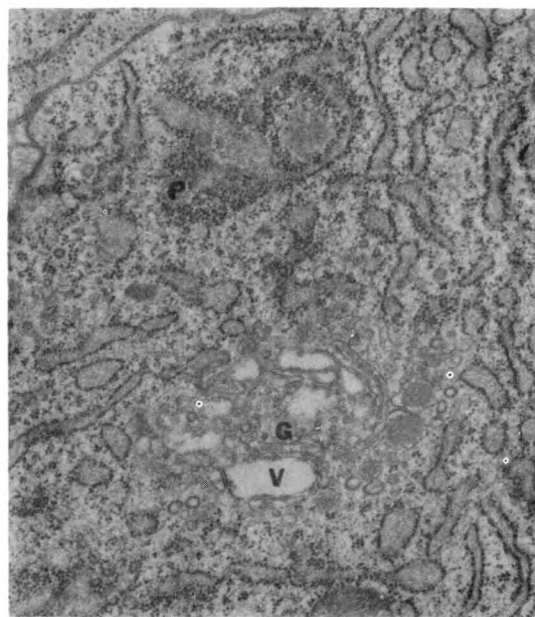


Fig. 4 Highly magnified ($\times 20\,000$) view of the Golgi region (G) of a plasma cell shows that normally well-defined lamellae have lost their parallel orientation, and large vacuoles (V) appear irregularly dilated. That this is not an artefact of fixation can be readily appreciated by the well-preserved endoplasmic reticulum and whirls of polyribosomes (P).

showed hypoplasia coupled with histiocytic infiltration (Fig. 5). Electron microscopy examination showed the histiocytes to be large, relatively electron-dense, phagocytic cells with modest amounts of isolated ribosomes. These cells were actively phagocytic and contained debris consisting of dense inclusions and myelin figures (Fig. 6).

CONTROL LYMPH NODES FROM RABBITS
INFECTED WITH BCG OR
STAPHYLOCOCCUS AUREUS

The lymph nodes of rabbits challenged at multiple sites at the back with *Staph. aureus* or *Mycobacterium tuberculosis* var. *bovis* (BCG) were different from those of syphilitic animals; neither contained the well-developed germinal centres with plasma cells that so typically characterised syphilitic lymph nodes. Nodes from animals infected with *Staph. aureus* showed modest primary nodules; BCG nodes were essentially normal. Lymph nodes infected with both *Staph. aureus* and BCG showed modest paracortical hyperplasia in direct contrast to the hypoplasia seen in rabbits infected with *T. pallidum*.

Discussion

Plasma cells have been shown to be present in germinal centres of lymph nodes from syphilitic patients based on identification of pyroninophilic cells by light microscopy examination (Turner and Wright, 1972). In the present study plasma cells were definitively identified as a prominent component of germinal centres in experimental syphilis in the rabbit. Electron microscopy examination also

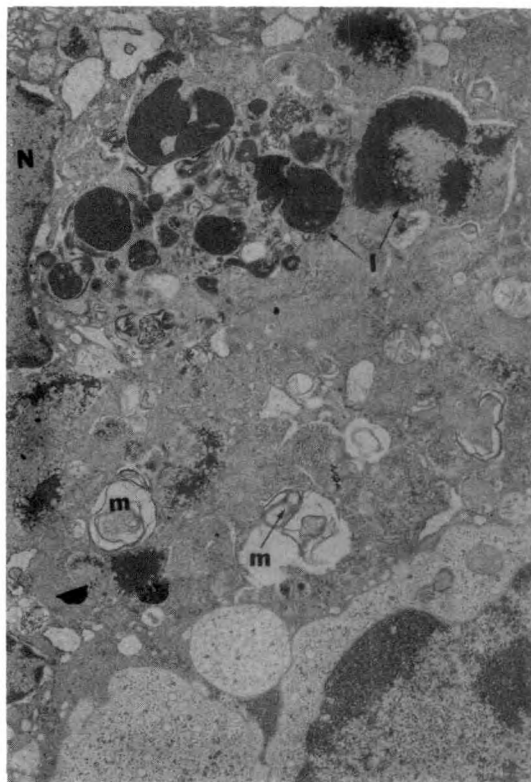


Fig. 6 Electron micrograph of a section through one portion of a histiocyte. The nucleus (N) is seen at the upper left-hand margin. Areas from two lymphocytes are seen in the lower right-hand corner. The histiocyte is typically phagocytic with many dense inclusions (I) and myelin figures (m) (magnification $\times 4800$).

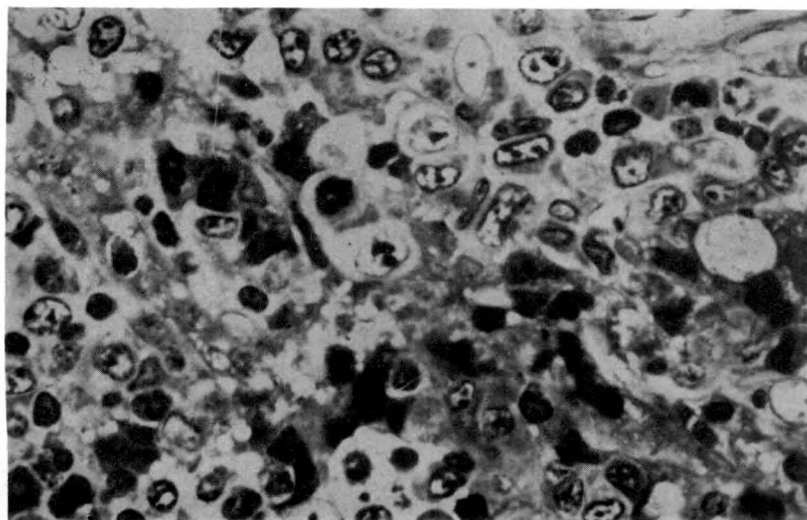


Fig. 5 Light micrograph showing histiocytic infiltration of the hypoplastic paracortex (magnification $\times 950$).

showed a variety of abnormalities in these cells, including lobulated nuclei, multiple nucleoli, and peculiarities in the Golgi region. These changes are most unusual. Since multiple nucleoli are seen only in immature precursors their persistence in plasma cells which have well-developed cytoplasmic endoplasmic reticulum may reflect asynchrony in cellular development. Abnormalities of the Golgi region were similar to those which have recently been shown to accompany an arrest of immunoglobulin synthesis (Tartakoff and Vassalli, 1977). The presence of these unusual cytoarchitectural features is of great interest in the light of the recent demonstration that the IgG response to a thymus-dependent heterologous antigen is nearly obliterated in early syphilis (Baughn and Musher, 1978).

The changes in the paracortex are in agreement with several previous reports which have demonstrated paracortical hypoplasia in syphilis (Festenstein *et al.*, 1967; Levene *et al.*, 1971; Turner and Wright, 1972). In addition to confirming the presence of histiocytes the present study shows them to be actively phagocytic. It is interesting to speculate as to whether phagocytic histiocytes are responsible for paracortical atrophy or whether they appear as a result of other factors which have brought about this atrophy. Treponemes were not detected in these histiocytes; however, treponemal antigen could also be responsible for histiocytic infiltration. In any case, hypoplasia of the paracortex, a thymus-dependent area, may be the anatomical correlate of suppressed cellular immunity which occurs during early syphilis (Levene *et al.*, 1969; Musher *et al.*, 1974; Friedman and Turk, 1975; Musher *et al.*, 1975, 1977; Pavia *et al.*, 1977). The presence of hypoplasia together with histiocytic infiltration is not unlike the changes observed in lymph nodes of patients with lepromatous leprosy (Turk, 1976), a disease which is also characterised by suppression of thymus-mediated immune function.

The authors are indebted to Ms Mona Thomas for secretarial assistance and to the Medical Media Service of the Veterans Administration Hospital for assistance with the illustrations.

The work was supported by NIH grant no. USPH AI 12618 03/05 and research funds from the Veterans Administration Hospital.

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Treatment monitoring in syphilis using the autoanalyser

D. E. MACFARLANE AND A. MCMILLAN

From the City Laboratory and Department of Venereology, Glasgow

SUMMARY Treatment monitoring in syphilis using the automated Reiter protein complement fixation test and the automated reagin test was investigated over a two-year period. Clearly defined response patterns were demonstrated in primary and secondary syphilis and, to a lesser extent, in latent syphilis, thus facilitating the assessment of treatment and the identification of treatment failures and reinfection. No obvious treatment failures were detected in those patients receiving penicillin, but two failures were noted in a group of patients with secondary syphilis treated with doxycycline. The combination of two automated tests overcame some of the disadvantages inherent in the use of a single reagin test.

Introduction

The Technicon Autoanalyser has been used in this laboratory since 1973 to perform screening tests for syphilis. For the first two years the reporting format used with the manual tests was followed, and the results of the automated Reiter protein complement fixation test (ARPCFT) were given as positive or negative. This policy was changed in 1975, and quantitative results were reported for both the ARPCFT and the automated reagin test (ART). Clearly, monitoring antibody levels with two different serological tests gave important additional information regarding response to treatment and stage of infection. We, therefore, decided to analyse the previous two years' results from two of the local clinics.

Material and methods

The automated tests were performed according to methods previously described (MacFarlane *et al.*, 1976).

Records were examined for a period of 24 months, and those patients with a sufficient number of antibody determinations were selected for analysis and classified as having primary, secondary, early latent (in the first two years), or late latent syphilis. Most of these patients had been treated with procaine

penicillin, 600 000 IU daily for 10 days (in cases of primary syphilis, group 1) or for 14 days (in cases of secondary syphilis, group 2; and latent syphilis, groups 3 and 4). A number of patients with secondary syphilis had been treated with doxycycline, 100 mg eight hourly for 14 days (group 5). Thus we were able to compare the two methods of treatment.

Results

GROUP 1

The serological records of 21 patients with primary syphilis are shown in Table 1. Before treatment 83% had positive ART readings and 95% had positive ARPCFT results (Fig. 1). (For the purpose of contracting Figures 1-6 any serology result which became negative during the course of treatment was presumed to remain negative unless subsequent test results proved otherwise.) Four months later all ART results were negative, and by six months both serological tests gave negative results in all cases. Before treatment the mean ART titre was 1/7.3, and the mean level of Reiter antibody was 2036 absorbance units (au) (100 au = antibody detectable at a dilution of 1/4); these figures declined rapidly after treatment to 1/1.5 and 691 respectively at eight weeks (Figs. 2 and 3). Low levels of Reiter antibody were still detectable at four and five months (Fig. 3).

GROUPS 2 AND 5

The serological records of 11 patients with secondary syphilis treated with penicillin (group 2) and 10 patients with secondary syphilis treated with doxy-

Address for reprints: Dr D. E. MacFarlane, The City Laboratory, 23 Montrose Street, Glasgow G1 1RN

Received for publication 22 June 1978

Table 1 Serological response to treatment with penicillin in 21 patients with primary syphilis (group 1)

Case no.	Interval after treatment (in months)																	
	Before treatment		1		2		3		4		5		6		9		12	
	ART*	ARPCFT†	ART	ARPCFT	ART	ARPCFT	ART	ARPCFT	ART	ARPCFT	ART	ARPCFT	ART	ARPCFT	ART	ARPCFT	ART	ARPCFT
1	16	8320	16	10240	16	7400	2	1296										
2	16	8960	4	1600		400												
3	8	1460	N	300														
4	2	1600		528														
5	4	29	2	1664		68												
6	32	8960	16	5000	4	664												
7	N	2400		68														
8	2	1408	N	2560		72												
9	N																	
10	2	480																
11	N	87	8	180		116		69										
12	4	90	16	1360	4	600												
13	32	2688			2	440												
14	8	880	8	43														
15	4	200	2															
16	8	2656	4	520		41												
17	8	1500	4	990	N	50												
18		485				40												
19	4	387																
20		50	2	3328		472		95										
21		120		63														

* ART results expressed as reciprocal of titre

† ARPCFT results expressed in absorbance units (au) (100 au = antibody detectable at a 1/4 dilution)

N = positive result in neat serum only

— Negative

Table 2 Serological response to treatment with penicillin in 11 cases of secondary syphilis (group 2)

Case no.	Interval after treatment (in months)																	
	Before treatment		1		2		3		4		5		6		9		12	
	ART*	ARPCFT†	ART	ARPCFT	ART	ARPCFT	ART	ARPCFT	ART	ARPCFT	ART	ARPCFT	ART	ARPCFT	ART	ARPCFT	ART	ARPCFT
32	16	6500	8	3200			2	71	N	1050	2	62	2	1200				
33	64	7680			4	320												
34	128	6400	32	2048	4	1600												
35	8	1400	8	1600			N	800										
36	8	84																
37	32	500	8	1110														
38	32	4719	16	2680														
39	32	1120			4	35	2											
40	16	1248	8	1120	4	400			N									
41	64	2304	64	1980	16	2304	16	600	8	380	4	320	4	43				
42	64	2800	32	3520	8	2170	4	504	2	45								

* ART results expressed as reciprocal of titre

† ARPCFT results expressed in absorbance units (au) (100 au = antibody detectable at a 1/4 dilution)

N = positive result in neat serum only

— Negative

Table 3 Serological response to treatment with doxycycline in 10 cases of secondary syphilis (group 5)

Case no.	Interval after treatment (in months)									
	Before treatment	1	2	3	4	5	6	9	12	18
	ART* ARPCFT†	ART ARPCFT	ART ARPCFT	ART ARPCFT	ART ARPCFT	ART ARPCFT	ART ARPCFT	ART ARPCFT	ART ARPCFT	ART ARPCFT
22	4 680	4 70	—	—	—	—	—	—	—	—
23	16 1120	4 480	N	N	N	—	—	—	—	—
24	16 5248	4 2624	—	—	—	25	—	—	—	—
25	8 800	2 216	N	—	—	—	—	—	—	—
26	16 1792	—	—	—	—	—	16 6400	64	12 160	—
27	4 860	4 1120	N	N	—	—	—	—	—	—
28	16 5800	—	—	—	—	39	—	—	—	—
29	16 3840	—	—	—	—	—	—	—	—	—
30	16 1520	16 1280	—	—	—	—	—	—	—	—
31	16 3840	8 3040	—	—	N 53	—	16 2240	—	—	—

* ART results expressed as reciprocal of titre
 † ARPCFT results expressed in absorbance units (au) (100 au = antibody detectable at a 1/4 dilution)

N = positive result in neat serum only
 — Negative

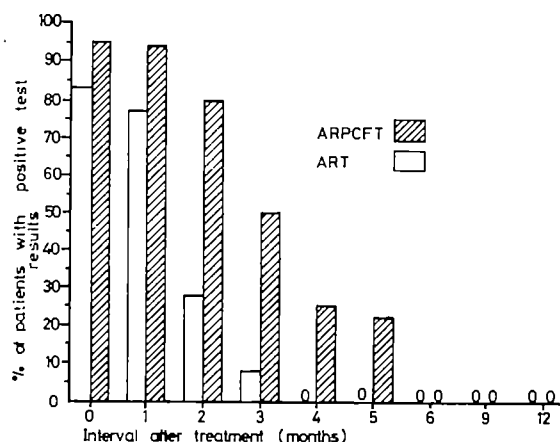


Fig. 1 Results of ARPCFT and ART for 21 patients with primary syphilis treated with penicillin (group 1)

cycline (group 5) are shown in Tables 2 and 3 respectively. Both tests gave positive results in all cases before treatment. In group 2, the ART and ARPCFT results were positive for eight weeks after the start of treatment, but by six months the ART and the ARPCFT results were negative in 57% and 71% of cases respectively (Fig. 4). By nine months all results were negative. Before treatment the mean ART titre was 1/42.2 and the mean level of Reiter antibody was 3160 absorbance units (Figs. 2 and 3). A sharp fall in ART titre occurred in the first eight weeks after treatment followed by a more gradual decrease over the next four months (Fig. 2). The mean Reiter antibody response followed a similar pattern with a sharp fall during the first three months and a more gradual decline to zero at nine months (Fig. 3).

In the group treated with doxycycline (group 5) both tests gave positive results before and for four weeks after treatment (Fig. 5). At five months 50% of the patients had no Reiter antibody and all ART results were negative. Twelve months after treatment all serological tests gave negative results. The mean ART titre before treatment was 1/12 with a corresponding figure of 2550 au for the ARPCFT. The ART titre had fallen to 1/1.5 after eight weeks and reached zero at five months (Fig. 2). There was a more gradual fall in Reiter antibody to 58 au at four months and thereafter low levels persisted until nine months (Fig. 3). Two treatment failures occurred in group 5. One patient (Case 26; Table 3) defaulted after his initial visit and was not seen for a period of six months. Serological tests at six months showed a static ART titre of 1/16 and a rise in Reiter antibody from 1792 au to 6400 au (Table 3). This patient failed to return to the clinic for a further three months, by which time his ART titre had risen to 1/64 and

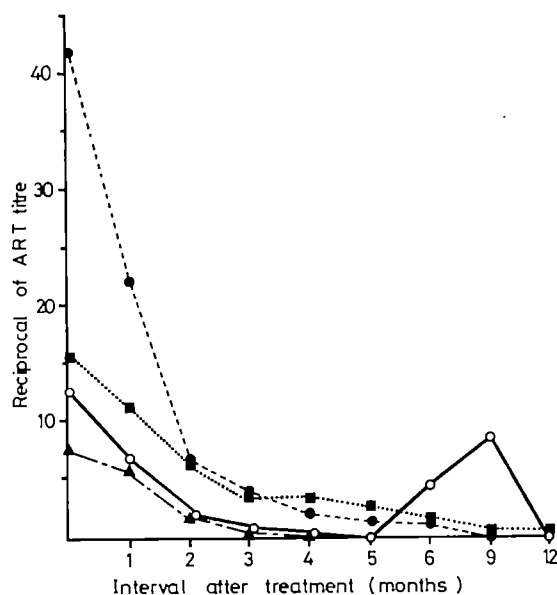


Fig. 2 Mean ART results before and after treatment in four patient groups. (The peak at nine months in group 5 represents treatment failures.)

▲—▲ group 1 (primary syphilis treated with penicillin); ●—● group 2 (secondary syphilis treated with penicillin); ■—■ group 3 (early latent syphilis treated with penicillin); ○—○ group 5 (secondary syphilis treated with doxycycline)

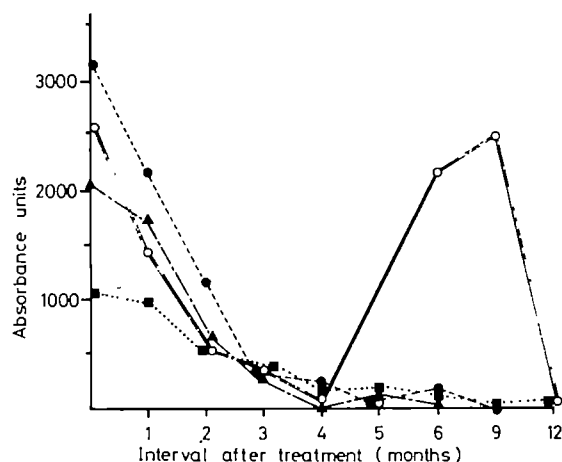


Fig. 3 Mean ARPCFT results before and after treatment in four patient groups. (The peak at nine months in group 5 represents treatment failures.)

▲—▲ group 1 (primary syphilis treated with penicillin); ●—● group 2 (secondary syphilis treated with penicillin); ■—■ group 3 (early latent syphilis treated with penicillin); ○—○ group 5 (secondary syphilis treated with doxycycline)

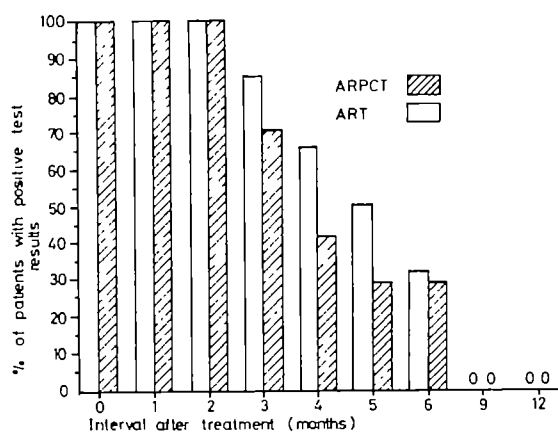


Fig. 4 Results of ARPCFT and ART for 11 patients with secondary syphilis treated with penicillin (group 2)

Reiter antibody levels to 12 160 au. One patient (Case 31; Table 3) appeared to be responding adequately to treatment, the ART titre falling from 1/16 to zero by three months and Reiter antibody falling from 3840 au to 53 au by four months, but by six months both antibody levels had climbed to pre-treatment levels. Both of these cases were assessed clinically as treatment failures rather than as reinfections.

GROUP 3

The serological records of eight patients with early latent syphilis (group 3) are shown in Table 4 and Fig. 6. The mean ART titre before treatment was 1/15.75 and the mean Reiter antibody level was 1079 au (Figs. 2 and 3). The antibody levels fell gradually over a period of six months and then tended to persist at low levels.

GROUP 4

Of the four patients with late latent syphilis (group 4) fluctuating antibody levels were clearly evident in three, and this makes any assessment of response to treatment very difficult (Table 4).

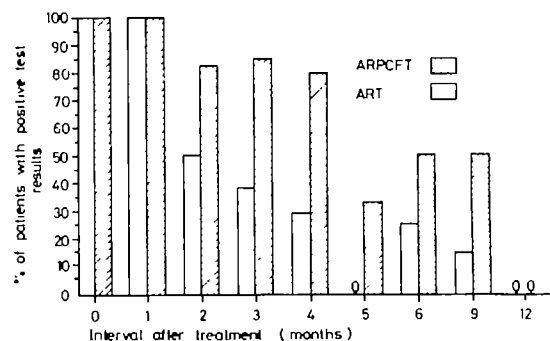


Fig. 5 Results of ARPCFT and ART for 10 patients with secondary syphilis treated with doxycycline (group 5)

Table 4 Serological response to treatment with penicillin in cases of early and late latent syphilis (groups 3 and 4 respectively)

Case no. and diagnosis	Interval after treatment (in months)											
	Before treatment	1	2	3	4	5	6	9	12	18		
	ART* ARPCFT†	ART ARPCFT	ART ARPCFT	ART ARPCFT	ART ARPCFT	ART ARPCFT	ART ARPCFT	ART ARPCFT	ART ARPCFT	ART ARPCFT	ART ARPCFT	ART ARPCFT
Early latent syphilis												
43	32	960	16	920	16	280	8	200	4	268	8	480
44	16	1248	16	790	16	1408	4	1536	4	300	4	70
45	2	88	4	82	4	—	—	—	—	—	—	—
46	—	67	—	—	—	66	—	—	—	—	—	—
47	32	3840	16	3000	2	650	—	150	—	—	—	—
48	32	1040	16	1024	8	536	—	—	—	—	—	—
49	8	590	—	—	—	—	—	—	—	—	—	—
50	4	800	8	954	4	912	4	560	4	65	—	—
Late latent syphilis												
51	2	800	4	1232	—	—	4	480	2	660	2	65
52	—	92	—	—	—	200	—	300	—	—	—	—
53	2	80	2	90	—	—	2	296	2	120	2	64
54	—	64	—	30	—	—	—	—	—	—	—	—

* ART results expressed as reciprocal of titre

† ARPCFT results expressed in absorbance units (au) (100 au = antibody detectable at a 1/4 dilution)

N = positive result in neat serum only

— Negative

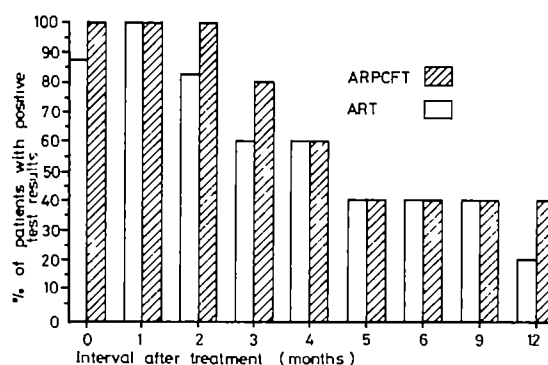


Fig. 6 Results of ARPCFT and ART in eight patients with early latent syphilis treated with penicillin (group 3)

Discussion

The diminishing antibody levels in patients with primary syphilis after treatment with procaine penicillin followed previously reported patterns (Wilkinson, 1970), the ART giving negative results by four months and the ARPCFT by six months (Fig. 1).

There were some interesting differences in the two groups of patients with secondary syphilis (groups 2 and 5). In the group treated with penicillin (group 2) the percentage of cases with positive ARPCFT results declined more rapidly than that of those with positive ART results (Fig. 4). This finding agrees with a previous observation by Schofield (1975). In the group treated with doxycycline (group 5) the position was reversed, with the positive ART results declining more rapidly (Fig. 5). In both tests antibody levels in group 5 declined to zero earlier than those in group 2 (Figs. 2 and 3). These differences in response to treatment can probably be attributed to the higher initial antibody levels in patients in group 2 (Figs. 2 and 3).

The two treatment failures with doxycycline recorded in group 5 (Table 3) are in clear contrast to the success of penicillin in the treatment of patients with primary and secondary syphilis; those with latent syphilis are more difficult to assess. It was assumed that these treatment failures were the result of incomplete courses of treatment. It is, however, possible that treatment with doxycycline would be more liable to failure as doxycycline is bacteriostatic and not bacteriocidal.

On the basis of our results, successful treatment will give negative serological results in both tests, after three to six months in cases of primary syphilis and after three to nine months in cases of secondary syphilis. It is possible that low levels of Reiter antibody (<100 au) may persist for some time in a few cases, but the large number of defaulters in this

series prevents any accurate observations on this matter.

In the patients with early latent syphilis (group 3) there was a gradual reduction in antibody levels for six months after treatment, with a tendency for low levels to persist thereafter in some cases. The Reiter antibody levels in this group before treatment were lower than those recorded in patients with primary and secondary syphilis.

Fluctuations in antibody levels are evident in three of the four patients with late latent syphilis (group 4), and there is no serological evidence of a definite response to treatment. In experimental infections in rabbits (Frazier *et al.*, 1952) treponemes were detected at intermittent intervals in the blood in cases of latent syphilis. The fluctuating antibody levels in latent syphilis seem to provide serological evidence for a similar intermittent bacteraemia in man.

For many years treatment monitoring has consisted of measuring cardiolipin antibody levels with the Venereal Disease Research Laboratory (VDRL) slide test or its carbon antigen equivalents. Such a system has several drawbacks. The reagin test results may remain negative (Cases 18 and 21; 52 and 54) or become fleetingly positive (Cases 46, 4, 7, 10, and 20). The change in reagin antibody levels may be delayed or occur at insignificant levels. This can be seen in Cases 9, 14, 22, and 44. It is particularly obvious and of some importance in one of the treatment failures (Case 26). The doubling-dilution method of measuring antibody levels is so crude and liable to error that only a pronounced rise or fall can be regarded as significant.

This investigation has brought to light several advantages in using the ARPCFT to monitor treatment. The more precise measurement system gives more accurate and reliable antibody estimations. The sensitivity of the ARPCFT allows detection and monitoring of very low antibody levels at all stages of infection, including early primary syphilis where it is one of the first tests to give positive results.

The serological results in cases of untreated, early primary syphilis are not always predictable. This is best illustrated by the results of a recent group of cases of early, primary syphilis (Table 5). In four of

Table 5 Initial serological result in six recent cases of early primary syphilis

Case no.	Serological test results		
	FTA-ABS‡	ARPCFT†	ART*
55	++	—	1/4
56	+/-	60	—
57	+	120	—
58	+/-	60	1/2
59	++	63	—
60	+	43	—

*ART results expressed as reciprocal of titre

†ARPCFT results expressed in absorbance units (au) (100 au = antibody detectable at a 1/4 dilution)

‡FTA results from borderline +/- to strongly positive ++++

these cases the ART gave negative results, in one case the ARPCFT result was negative, and in two cases the FTA-ABS test result was not positive. In our experience the FTA-ABS test and the ARPCFT are the two tests whose results become positive first in cases of early syphilis closely followed by the ART. It is, however, important to realise that any permutation of these three tests may combine to give positive or negative results in cases of early primary syphilis.

Treatment monitoring using both tests tends to overcome any disadvantages inherent in the use of a single reagin test and acts as a dual checking system on all results.

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Rapid presumptive diagnosis of gonococcal urethritis in men by the limulus lysate test

VINCENT A. SPAGNA, RICHARD B. PRIOR, AND ROBERT L. PERKINS

From the Division of Infectious Diseases, Department of Medicine, The Ohio State University College of Medicine, Columbus, Ohio, USA

SUMMARY In an evaluation of the limulus assay as a method for detecting endotoxin in urethral exudates, positive results for urethral samples at a 1/200 dilution were obtained from 73 out of 73 patients with culture-positive gonococcal urethritis while negative results were obtained from 26 out of 27 patients with culture-negative urethral specimens. A specimen from one patient, which gave negative results on Gram stain and culture, gave positive results to the limulus test. The overall accuracy of the limulus test for predicting culture results was 99% ($P < 0.001$). Thus, in preliminary studies of otherwise healthy men, the results of the limulus assay correlated with those of biological methods for diagnosing urethral gonorrhoea; the test may, therefore, be of use in identifying cases of nongonococcal urethritis.

Introduction

Studies in recent years (Melton, 1976; Felman and Scaffidi, 1977) have shown that gonorrhoea is the most common, reportable infectious disease in the United States. In 1976, there were one million reported cases of gonorrhoea (Venereal Disease Control Division, 1976), over 600 000 occurring in men. Rapid and sensitive diagnostic methods are needed in the management of patients with gonococcal and nongonococcal urethritis (NGU). A variety of techniques for diagnosing gonococcal disease have been developed (Welch and O'Reilly, 1973; Jacobs and Kraus, 1975; Feng *et al.*, 1977) but most are complex and generally require unavailable materials.

Pioneer studies (Levin and Bang, 1964) showed that a lysate from washed amoebocytes of the *limulus polyphemus*, the horseshoe crab, formed a gel in the presence of minute amounts of endotoxin elaborated by Gram-negative bacteria. The limulus assay has been used for detecting endotoxin in blood (Levin *et al.*, 1970), urine (Jorgensen *et al.*, 1973), and joint and spinal fluids (Tuazon *et al.*, 1977), and as a method for pyrogen testing of injectable commercial fluids (Jorgensen and Smith, 1973). The use of the limulus endotoxin assay in the diagnosis of

gonococcal urethritis and as a possible means of identifying cases of NGU is the subject of this report.

Material and methods

STUDY POPULATION

One hundred men with uncomplicated urethritis seen at the Columbus Health Department Venereal Disease Clinic were studied. These patients had sought treatment because of urethral discharge or dysuria or both and were selected on a random basis; only those with a purulent urethral discharge or a discharge obtained after urethral massage were included. Patients receiving antibiotic therapy within 10 days of presentation were excluded. The mean age was 23.6 years and the range, 19-28 years. The patients presented one to nine days after noticing symptoms, with a mean of 2.8 days.

DIAGNOSTIC PROCEDURES

Each patient underwent a standardised interview, concerning demography, sexual and venereal disease histories, and present illness, and an examination of the genitals and inguinal lymph nodes.

Urethral exudates (0.05-0.1 ml) were obtained from the urethral meatus by gentle aspiration with a tuberculin syringe (without needle) and transferred to a pyrogen-free plastic test tube containing 1 ml of pyrogen-free water. All specimens were frozen at -20°C before being tested by the limulus assay.

Address for reprints: Dr Vincent A. Spagna, Ohio State University Hospital, 410 West 10th Avenue, Columbus, Ohio 43210, USA

Received for publication 15 August 1978

For culture purposes, a sterile, calcium alginate-tipped, wire, urethrogenital swab (Wilson Diagnostics) or sterile, bacteriological loop was passed 3-4 cm into the urethra. The samples were streaked directly on to Thayer-Martin media and incubated for 48 hours at 35°C in 5% carbon dioxide. No cultures for viruses or chlamydiae were carried out.

The same procedure was repeated to obtain a specimen for Gram-staining and microscopical examination.

LABORATORY METHODS

Gram-stained smears of urethral exudate were examined under $\times 1000$ magnification oil immersion for the presence of polymorphonuclear cells and Gram-negative diplococci. (One microscope was used by one observer.) The results of the smears were considered positive if typical Gram-negative diplococci were seen, whether located intracellularly or extracellularly. A presumptive identification of isolates of *Neisseria gonorrhoeae* was based on characteristic colonial morphology, positive oxidase test, and the presence of Gram-negative diplococci. Isolates were further identified as *N. gonorrhoeae* by typical sugar fermentation reactions. The microscopical diagnosis of NGU required a mean of 10 leucocytes per $\times 1000$ field in a least five fields and the absence of Gram-negative diplococci.

LIMULUS ASSAY

Frozen specimens, of urethral exudate were thawed, vortexed, and diluted in pyrogen-free water to final dilutions of 1/20 and 1/200 of the original sample; 0.2 ml of the diluted specimens was added to single-test Pyrotest™ vials (Difco, by courtesy of A. L. Lane), mixed incubated at 37°C in a water bath for one hour, and read. A firm opaque gel which remained adherent to the bottom of the vial when inverted through 180° was interpreted as a positive test result; the absence of firm gelation was interpreted as a negative result. Known positive and negative control samples were also tested with each assay. The limulus assays were read without previous

knowledge of the microbiological findings. The sensitivity of the limulus test was determined by the detection of less than 0.1 ng/ml *Escherichia coli* endotoxin.

ANALYSIS OF DATA

The results of the limulus assay, Gram-stained smears, and cultures were entered into a Hewlett-Packard Model 9825A programmable calculator for subsequent determination of correlation. Yates's χ^2 method of analysis was used for the determination of statistical significance.

Results

A comparison of the results of the limulus assay for specimens at a 1/200 dilution with those of the Gram-stained smears of patients with and without culture-positive gonococcal urethritis are shown in the Table. Of 73 patients with culture-positive gonorrhoea, 73 (100%) had positive limulus test results and 72 (99%) positive Gram-stained smear results; of 27 patients with probable nongonococcal urethritis, 26 (96%) had negative limulus test results and 27 (100%) negative Gram-stained smear results ($P < 0.001$). Testing of the exudates at a 1/20 dilution gave positive assay results in five (19%) of the 27 patients with nongonococcal urethritis. On retesting at 1/100 and 1/200 dilutions, all the specimens gave negative results to the limulus assay. Only one patient with a negative smear result and a negative culture result for *N. gonorrhoeae* had a positive result to the limulus assay at a 1/200 dilution; on retesting, this specimen produced a positive result at a 1/800 dilution.

Discussion

The outer membranes of *N. gonorrhoeae* are known to contain protein, lipopolysaccharide, and loosely bound lipids (Johnston and Gotschlich, 1974; Wolf-Watz *et al.*, 1975). Recently, Rice and Kasper (1977) have shown that gelation of limulus lysate was caused by the intact outer membranes of *N. gonorrhoeae* and each of its constituents at concentrations as low

Table Results of Gram-stained smears and limulus assay for patients with culture-positive gonococcal and nongonococcal urethritis

Diagnosis	No. of patients	Gram-stained smears		Limulus assay*	
		Positive	Negative	Positive	Negative
Gonococcal urethritis	73	72	1	73	0
Nongonococcal urethritis	27	0	27	1	26

*Original sample diluted 1/200

as 0.7 ng/ml of intact outer membrane, 0.08 ng/ml of protein fraction, and 0.16 ng/ml of lipopolysaccharide fraction. Thus, with the high sensitivity of the limulus assay for components of *N. gonorrhoeae* false-negative results for urethral samples would be unlikely to occur in cases of uncomplicated gonococcal urethritis. Our findings support this hypothesis. In those patients with culture-positive gonococcal urethritis dilutions of 1/200 of the urethral samples produced positive limulus reactions consisting of solid, opaque gelation in every instance.

The potential aetiological agents in NGU have been the focus of recent intense bacteriological, virological, and serological studies (Holmes *et al.*, 1975). The hypothesis that *Chlamydia trachomatis* and *Ureaplasma urealyticum* are the most common urethral pathogens in NGU has been supported by considerable evidence (Wong *et al.*, 1977; Bowie, 1978). *Corynebacterium genitalium* type 1 (Furness *et al.*, 1977), and other bacteria (Bowie *et al.*, 1977), herpesvirus hominis (Jeansson and Molin, 1960), and *Trichomonas vaginalis* (Catterall, 1960) have also been implicated as possible aetiological agents in NGU. Although documented evidence is lacking, it is possible that within the spectrum of agents associated with NGU some could elaborate substances which would produce positive limulus test results. The fact that *C. trachomatis* has a cell wall with antigenic properties similar to but not identical with that of Gram-negative bacteria (Kuo *et al.*, 1977) supports that possibility. Compounds other than bacterial endotoxins (Elin and Wolff, 1973; Wildfeuer *et al.*, 1974) can also give positive results to the limulus test.

Our observations indicated that such potential substances existed in the exudates of some of our patients with NGU. With the exception of one case, however, dilutions of 1/200 of the urethral samples resulted in negative limulus reactions. One specimen showed no bacterial forms on a Gram-stained smear, was culture-negative for *N. gonorrhoeae*, and produced a positive limulus test result at a dilution of 1/800. Further questioning of this patient with a false-positive result to the limulus test elicited that he had received antibiotic therapy for gonorrhoea on eight occasions in three years, had last been treated three months previously and had access to tetracycline. While the reasons for this discrepancy are speculative, the possibilities of sampling-error, unreported antibiotic usage, or contaminated (pyrogen-containing) equipment may provide an explanation.

In the present study, the results of the limulus test on diluted samples of urethral exudate correlated with those of bacteriological methods of diagnosis of urethral gonorrhoea in men with an overall accuracy of 99%. These preliminary studies of otherwise

healthy men with exudative urethritis suggest that the limulus lysate assay is rapid, reliable, and sensitive for the detection of *N. gonorrhoeae* and may assist in identifying cases of NGU. With this test as an adjunct to other diagnostic procedures, it may be possible to give specific treatment at the patient's initial visit even before culture results are available.

We are indebted to Dr James Curran, Dr Joseph Lossick, Dr Phum Si The, Ms Jeanne Gwynn, Ms Betty Huber, Mr Michael Smeltzer, Mr Courtland Storck, and Mr Paul Vangeloff of the Columbus Health Department Venereal Disease Clinic for their cooperation and assistance.

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Assessment of a selective medium for the isolation of *Neisseria gonorrhoeae*

ELIZABETH TAYLOR AND IAN PHILLIPS

From the Department of Microbiology, St Thomas's Hospital Medical School, London

SUMMARY Specimens of urethral pus from 312 men with gonorrhoea were diluted and inoculated on to non-selective and selective media, the latter containing vancomycin, colistin, nystatin, and trimethoprim. Although three (1%) isolates of *Neisseria gonorrhoeae* were inhibited by trimethoprim 4 mg/l and 10 by vancomycin 2 mg/l, only two strains failed to grow on a selective medium from large inocula but eight strains sensitive to vancomycin failed to grow on a selective medium from a light inoculum. These few failures do not appear to negate the value of the selective medium.

Introduction

Various antibiotics have been used in selective media for the isolation of the gonococcus from sites with a heavy, normal, or contaminating flora. We have used a medium containing vancomycin, colistin, and trimethoprim with success for a number of years (Phillips *et al.*, 1972). It has been suggested, however, that both vancomycin and trimethoprim inhibit a considerable number of strains of *N. gonorrhoeae* and are therefore unacceptable (Cross *et al.*, 1971; Brorson *et al.*, 1973). We, therefore, decided to investigate the *in-vitro* activity of trimethoprim and vancomycin on the gonococcus and to assess results in the light of the success or failure of primary isolation of the same strains on non-selective and selective media.

Materials and methods

ISOLATES OF *NEISSERIA GONORRHOEAE*

Samples of pus from male patients with gonorrhoea attending the Department of Genitourinary Medicine at St Thomas's Hospital were collected on a plain cottonwool swab, which was immediately placed in 1 ml of nutrient broth (Southern Group Laboratories) containing 10% saponin-lysed horse blood. Standard loopfuls (0.007 ml) of the neat broth (large inoculum) and a dilution of 1/1000 (small inoculum) were used to inoculate in parallel both a non-selective

and selective medium. The non-selective medium was lysed-blood agar (Oxoid DST agar CM 261 plus 5% saponin-lysed horse blood), and the selective medium was 10% lysed-blood agar (Difco heart infusion agar 0044-01) containing vancomycin 3 mg/l, colistin sulphomethate 100 units/ml, nystatin 12.5 units/ml, and trimethoprim 5 mg/l, similar (except in the agar base) to VCNT medium, which we have used for many years for the routine isolation of gonococci (Phillips *et al.*, 1972). We had previously shown that the two different media performed similarly when antibiotics were not incorporated, and we, thus, used different agar as described for purely operational reasons.

MINIMUM INHIBITORY CONCENTRATIONS

Minimum inhibitory concentrations (MICs) were determined for vancomycin and trimethoprim by a lysed-blood agar (Oxoid DST) dilution method described previously (Phillips *et al.*, 1970; Lawrence *et al.*, 1973).

The inoculum was prepared from isolates cultured on a non-selective medium and consisted of 10^4 – 10^5 colony-forming units.

Results

A total of 312 isolates was studied, comprising all those that grew on primary isolation on the non-selective medium during the period of study.

Table 1 shows the distribution of sensitivity of isolates to vancomycin and trimethoprim. Ten (3%) isolates were inhibited by vancomycin 2 mg/l or less and three (1%) by trimethoprim 4 mg/l. No isolate was highly sensitive to both agents.

Address for reprints: Professor I. Phillips, Department of Medical Microbiology, St Thomas's Hospital Medical School, London SE1 7EH

Received for publication 12 October 1978

Table 1 Susceptibility of 312 isolates of *Neisseria gonorrhoeae* to vancomycin and trimethoprim

Antibiotics	% of isolates with MICs (mg/l) of									
	1	2	4	8	16	32	64	128	>128	
Vancomycin	0.5	2.5	10	32	36	16	3			
Trimethoprim			1	2	19	43	22	8	5	

Table 2 shows the results of primary cultures of large and small inocula (the latter varying between about 10 and 100 colony-forming units) on non-selective and selective media. All three organisms with a MIC of 4 mg/l for trimethoprim grew from both large and small inocula on the selective medium. Among the remaining isolates two failed to grow even from large inocula while eight failed to grow and two were considerably inhibited from small inocula on the selective medium. Examination of the results for vancomycin shows that these failures were associated with vancomycin sensitivity. Two isolates with MICs of 1 mg/l for vancomycin completely failed to grow on the selective medium. A further eight isolates, with MICs of 2 mg/l, grew well from large inocula but either failed to grow completely (six isolates) or were very considerably inhibited (two isolates) from small inocula. All isolates with MICs of 4 mg/l or more for vancomycin grew well from large and small inocula, regardless of the MICs for trimethoprim.

Discussion

Selective media are vital for the microbiological diagnosis of gonorrhoea in women, but the assessment of the performance of these media is difficult because of the unreliability of direct Gram stains of material from the female genital tract and the lack, therefore, of a basis for comparison. Gram

stains of specimens of urethral pus from men are, in contrast, a very good means of diagnosing gonorrhoea, and samples from men, therefore, offer a better method of assessing the performance of selective media. The number of gonococci in specimens of urethral pus from men, however, is usually very large, and we, therefore, decided that a more stringent test of the inhibitory activity of a selective medium might be provided by the use of inocula prepared from diluted specimens of pus.

The other means of examining the probable effects of antibiotics in selective media is to determine the MICs of the antibiotics. Because the conditions suitable for sensitivity testing differ from those for isolation, discrepancies might be expected.

Our results show that gonococci that are sensitive to trimethoprim and vancomycin are uncommon in patients attending the Department of Genitourinary Medicine at St Thomas's Hospital. Only 3% of strains were sensitive to vancomycin 2 mg/l, a similar result to that of 9% of strains sensitive to vancomycin 3 mg/l in our last study (Phillips *et al.*, 1972), and only 1% were sensitive to trimethoprim 4 mg/l compared with 12% to trimethoprim 3 mg/l in our last study which shows a possible increase in resistance. Results of parallel primary isolation on non-selective and selective media, however, show that this degree of trimethoprim sensitivity did not prevent the isolation of *N. gonorrhoeae* from either large or small inocula of the few strains in this group. Vancomycin sensitivity was important, however, in that both of the isolates that were highly sensitive (MIC=1 mg/l) failed to grow on the selective medium, while eight slightly less sensitive isolates (MIC=2 mg/l) either failed to grow or were considerably inhibited in small inocula, although they grew perfectly well from larger inocula. It is of interest that only one of the isolates out of the 312

Table 2 Results of parallel cultures of 312 isolates of *N. gonorrhoeae* on non-selective and selective media containing vancomycin 3 mg/l and trimethoprim 5 mg/l

			<i>No. of isolates giving growth on media</i>			
			<i>Non-selective</i>	<i>Selective</i>		
<i>Antibiotic</i>	<i>MIC (mg/l)</i>	<i>Inoculum</i>		++	+	-
Trimethoprim	4	large and small	3	3	0	0
	8	large	309	307	0	2
		small	309	299	2	8
Vancomycin	1	large and small	2	0	0	2
	2	large	8	8	0	0
		small	8	0	2	6
	4	large and small	302	302	0	0

+ + Same amount of growth as on non-selective medium

+ Less growth than on non-selective medium

- No growth

was not detected in the routine diagnostic section of our laboratory, which uses selective plates inoculated in the clinic with undiluted specimens of urethral pus. These results contrast with those of Cross *et al.* (1971), who found that 8% of primary isolations failed, and of Brorson *et al.* (1973), who lost 10% of primary isolations because of the high level of sensitivity to vancomycin among gonococci.

Thus, the use of our selective medium, consisting of heart-infusion agar containing 10% lysed horse blood, vancomycin 3 mg/l, and trimethoprim 5 mg/l in addition to colistin and nystatin (VCNT), seems to be fully justified in our clinic for the isolation of gonococci from men and, by inference, from women.

We thank Dr C. S. Nicol and the members of his department for their co-operation in this study, which was made possible by a DHSS grant.

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Comparison of anal and rectal swabs in the diagnosis of anorectal gonorrhoea in women

BARBARA KOLATOR* AND PHILIP RODIN†

*From the *Venereal Diseases Reference Laboratory and the †Whitechapel Clinic, The London Hospital, London*

SUMMARY Among 228 women with gonorrhoea (confirmed by culture), swabs taken blindly from the anal canal gave positive results in 26·3% and those taken from the rectum under direct vision in 27·6%. Swabs from both sites gave positive results in 23·2% of patients, from the anal canal alone in 3·1%, and from the rectum alone in 4·4%. Thus, culture of anal canal swabs seems to give as reliable results as rectal swabs when proctoscopy cannot be carried out.

Introduction

Rectal swabs for microscopical examination and culture are taken routinely from women in only a few clinics in England and Wales. Even in the investigation of contacts of gonorrhoea rectal swabs are taken in only 20% of clinics (Adler, 1978). The rectum is commonly affected in women with gonorrhoea, and it may be the only site from which the organism is recovered in about 5% of cases. The subject was reviewed briefly by Bhattacharyya and Jephcott (1974).

Thus microscopy and culture of rectal swabs are important in detecting some cases of gonorrhoea. Furthermore, although the diagnosis can usually be made by isolating the organism from other sites, it is necessary to prove that any associated rectal infection has also responded to treatment (Schroeter and Reynolds, 1972).

Although examination by proctoscopy is preferred, because it allows a clinical assessment to be made and material for culture to be selected under direct vision, not all patients agree to this examination. Reports on the success of taking anal swabs for the isolation of gonococci have varied. Bhattacharyya and Jephcott (1974), in a small series of female patients, found that anal swabs gave fewer positive results than rectal swabs in the detection of gonorrhoea. Deheragoda (1977), however, obtained almost as good results with anal swabs as with rectal swabs in male homosexuals. In the present study we

compare the results from anal and rectal specimens in a larger series of female patients.

Material and methods

As well as routine examination of cervical and urethral specimens, rectal specimens for Gram staining and culture were taken via a proctoscope from all new female patients attending the Whitechapel Clinic of the London Hospital. For the present study anal swabs were also taken. The swab was introduced blindly 2 or 3 cm into the anal canal and inoculated on to one half of a culture plate (brain heart infusion blood agar with 3 µg/ml vancomycin and 7·5 µg/ml colistin); the other half of the plate was reserved for inoculation of the rectal swab taken next under direct vision through a proctoscope. Organisms were identified as gonococci on the basis of their colonial appearance and microscopical morphology in Gram-stained smears, of positive results to the oxidase test, and of acid production from glucose but not maltose, lactose, or sucrose.

Results

During the course of the study gonorrhoea was confirmed by culture at one examination in 228 women (Table 1). The frequency of isolation from each site is shown in Table 1 and a comparison of the results from anal canal and rectal specimens is given in Table 2. The results of the Gram-stained smears for 228 women with culture-positive gonorrhoea are given in Table 3. Smears from anal canal specimens were not made.

Address for reprints: Dr P. Rodin, Whitechapel Clinic, The London Hospital, London E1 1BB

Received for publication 6 October 1978

Table 1 Site of positive isolation in 228 women with culture-positive gonorrhoea

No. of patients	No. of swabs giving positive results at each site							
	Urethra		Cervix		Anal canal		Rectum	
	No.	%	No.	%	No.	%	No.	%
228	203	89	218	95.6	60	26.3	63	27.6

Table 2 Comparison of results from anal canal and rectal specimens in 228 women with culture-positive gonorrhoea

Site of specimens giving positive results by culture							
Anal canal or rectum or both		Anal canal and rectum		Anal canal alone		Rectum alone	
No.	%	No.	%	No.	%	No.	%
70*	30.7	53	23.2	7	3.1	10	4.4

*In five (2.2%) the cervical and urethral cultures gave negative results

Table 3 Results of Gram-stained smears for 228 women with culture-positive gonorrhoea

Site of specimens giving positive results by Gram-staining						Total positive at one or more sites	
Urethra		Cervix		Rectum			
No.	%	No.	%	No.	%		
97	42.5	157	68.9	15	6.6	179	78.5

Discussion

The results show that cultures of anal canal specimens taken blindly give comparable results to those taken under direct vision through a proctoscope. Bhattacharyya and Jephcott (1974) found that culture of anal canal swabs in women gave less reliable results, but this might have been due to loss of a small inoculum in Stuart's transport

medium. Deheragoda (1977), using direct plating for male homosexuals, obtained similar results to ours.

We still take specimens for the diagnosis of gonorrhoea through a proctoscope and direct vision is essential when looking for evidence of non-specific proctitis. There are a few patients, however, who object to proctoscopy, and it would certainly be worth taking an anal swab in these cases.

Barlow and Phillips (1978) found that Gram-stained urethral smears contributed little to the diagnosis of gonorrhoea in women and suggested that they could be omitted from routine examination. These workers found that only 12.3% of urethral smears gave positive results; the results of the urethral smear enabled the diagnosis to be made at the first visit so that treatment could be started—on the basis of a positive result by urethral smear alone—in only 2.6% of cases. These results seem at variance with those of most other workers. In our study, urethral smears gave positive results in 42.5% of cases, and we were able to start treatment, when it was the only test giving positive results at the first visit, in 6.6% of our cases; however, the corresponding figure was only 0.9% with rectal smears.

We thank Dr A. E. Wilkinson, Director of the Venereal Disease Reference Laboratory, for his advice.

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Individual susceptibility to neisserial infection?

H. YOUNG, A. B. HARRIS, AND D. H. H. ROBERTSON

From the Department of Bacteriology, Edinburgh University Medical School, and the University Department of Venereology, Royal Infirmary, Edinburgh

SUMMARY Specimens from genital, anorectal, and pharyngeal sites from 1671 men and 1419 women were cultured for *Neisseria gonorrhoeae*. Pharyngeal specimens were also cultured for *Neisseria meningitidis*. *N. gonorrhoeae* was isolated from a genital site 2·7 times more often in men and 1·8 times more often in women who also carried meningococci in their pharynx than from those who did not; the meningococcus was isolated 3·4 times more often from men and 2·0 times more often from women with genital gonorrhoea than from those without. In both men and women the association of each organism with the other was statistically significant ($P < 0\cdot001$) and may be related to sexual behaviour rather than to individual susceptibility to neisserial infection.

Introduction

The gonococcus and the meningococcus, the two most important human pathogens in the genus *Neisseria*, are closely related genetically (Kingsbury, 1967; Kingsbury *et al.*, 1969) and antigenically (Tramont *et al.*, 1974). If there are specific host factors which increase the susceptibility of certain individuals to these organisms, their study could increase our knowledge of the pathogenicity of gonococcal and meningococcal infections.

In an investigation by Willcox *et al.*, (1977) of 150 cultures of throat exudates from patients (predominantly male) who were examined at a clinic for sexually transmitted diseases because they had a history of orogenital intercourse, the meningococcus was isolated nearly six times more often from those with genital gonorrhoea than from those without. In the same study the gonococcus was found 2·5 times more often in those who carried the meningococcus in the pharynx than in those who did not. These workers suggested (Willcox *et al.*, 1977) that confirmation of these findings would support the theory of individual susceptibility to the acquisition of neisseriae.

This paper reports our findings on all patients, male and female, from whom genital (and, in some cases, anorectal) and pharyngeal specimens were cultured during 1977.

Materials and methods

Specimens of genital and pharyngeal exudates obtained from 1671 men and 1419 women attending the Department of Venereology at the Royal Infirmary, Edinburgh, during 1977 were cultured in parallel for *Neisseria gonorrhoeae*. The pharyngeal specimens were also cultured for *Neisseria meningitidis*.

Urethral and pharyngeal specimens were taken from all male patients in the study while anorectal specimens were also taken for culture from homosexual men. Urethral, cervical, anorectal, and pharyngeal specimens were taken for culture from all female patients in the study. These patients comprised all known contacts of gonorrhoea. Patients were not routinely asked about recent oral contact, but if such a history was obtained they were included in the study.

Material was inoculated directly on to modified New York City medium (Young, 1978a) at the time of the patient's initial examination. After inoculation, plates were held at 36°C in a carbon dioxide-enriched (10%) atmosphere and transferred to the laboratory within four hours. Specimens were processed, and *N. gonorrhoeae* and *N. meningitidis* were identified by fluorescent antibody and rapid carbohydrate utilisation tests (Young, 1978b).

Statistical analysis was made by the χ^2 method with Yates's correction.

Results

The results of cultures from the 1671 male and 1419

Address for reprints: Dr H. Young, Department of Bacteriology, University Medical School, Teviot Place, Edinburgh EH8 9AG, Scotland

Received for publication 23 August 1978

female patients are shown in Tables 1 and 2 respectively.

The isolation of gonococci from genital sites is compared with that of meningococci in the pharynx of men and women in Tables 3 and 4 respectively; the isolation of meningococci from the pharynx compared with that of gonococci from genital sites is shown in Tables 5 and 6.

Table 1 *Isolation of Neisseria gonorrhoeae from genital and anorectal sites and of Neisseria meningitidis from the pharynx of 1671 men*

Genital/anorectal sites	Pharyngeal culture results for <i>N. meningitidis</i> *		
	Positive	Negative	Total
Culture results for <i>N. gonorrhoeae</i>			
Positive	89	262	351
Negative	97	1223	1320
Total	186	1485	1671

* χ^2 89.8; $P < 0.001$

Table 2 *Isolation of Neisseria gonorrhoeae from genital and anorectal sites and of Neisseria meningitidis from the pharynx of 1419 women*

Genital/anorectal sites	Pharyngeal culture results for <i>N. meningitidis</i> *		
	Positive	Negative	Total
Culture results for <i>N. gonorrhoeae</i>			
Positive	96	256	352
Negative	148	919	1067
Total	244	1175	1419

* χ^2 32.4; $P < 0.001$

Table 3 *Isolation of gonococci from genital and anorectal sites in relation to that of meningococci in the pharynx of 1671 men*

Pharyngeal cultures	No.	Genital and anorectal gonorrhoea	
		No.	%
Meningococci-positive	186	89	47.9
Meningococci-negative	1485	262	17.6
Total	1671	351	21.0

Table 4 *Isolation of gonococci from genital and anorectal sites in relation to that of meningococci in the pharynx of 1419 women*

Pharyngeal cultures	No.	Genital and anorectal gonorrhoea	
		No.	%
Meningococci-positive	244	96	39.3
Meningococci-negative	1175	256	21.8
Total	1419	352	24.8

Table 5 *Isolation of meningococci from the pharynx in relation to that of gonococci from genital and anorectal sites in 1671 men*

Genital/anorectal cultures	No.	Pharyngeal cultures showing meningococci	
		No.	%
Gonococci-positive	351	89	25.4
Gonococci-negative	1320	97	7.4
Total	1671	186	11.1

Table 6 *Isolation of meningococci from the pharynx in relation to that of gonococci from genital and anorectal sites in 1419 women*

Genital/anorectal cultures	No.	Pharyngeal cultures showing meningococci	
		No.	%
Gonococci-positive	352	96	27.3
Gonococci-negative	1067	148	13.9
Total	1419	244	17.2

The gonococcus was isolated 2.7 times more often from men and 1.8 times more often from women who were pharyngeal carriers of meningococci than from those who were not (Tables 3 and 4). Conversely, meningococci were isolated from the pharynx 3.4 times more often from men and 2.0 times more often from women with gonorrhoea than from those without (Tables 5 and 6). The association of each organism with the other is highly significant, in both men (χ^2 89.08; $P < 0.001$) and women (χ^2 32.4; $P < 0.001$).

The incidence of pharyngeal gonorrhoea in our study was 5.4% (19/352) in women and 3.1% (11/351) in men with genital or anorectal gonorrhoea or both; this represents an incidence of 1.3% (19/1419) and 0.7% (11/1671) of all female and male patients respectively.

Discussion

Our results confirm the earlier finding (Willcox *et al.*, 1977) that individuals who carry the meningococcus in the pharynx and who have been exposed to the risk of gonorrhoea are more likely to yield positive results to genital culture for *N. gonorrhoeae* and vice versa. Although in the earlier study (Willcox *et al.*, 1977) the meningococcus was isolated approximately six times more often from patients with genital gonorrhoea than from those without, the corresponding factors in our study are lower, 3.4 for men and 2.0 for women. The former six-fold difference (Willcox *et al.*, 1977) reflects a lower meningococcal carriage rate in the group without gonorrhoea (5.3%) and a higher rate in the group



with genital gonorrhoea (31.3%) than the corresponding carriage rates in our study—7.4% and 13.9% in men and women without gonorrhoea and 25.4% and 27.3% in those with gonorrhoea respectively. Our finding that the gonococcus was isolated 2.7 times more often from men and 1.8 times more often from women who were pharyngeal carriers of the meningococcus than from those who were not, is in good agreement with the 2.5-fold difference quoted previously (Willcox *et al.*, 1977).

Thus, in our study each organism was isolated two to three times more frequently in the presence of the other. The factors responsible for this association are unknown. It has been reported (Foster and Labrum, 1976) that there is an increased risk of gonococcal infection in blood group B subjects, and other workers (Miler *et al.*, 1977) confirmed this for white patients but not for West Indians. It was concluded (Miler *et al.*, 1977) that such data must be carefully collected on well-defined ethnic groups over a range of samples before a definite conclusion on intrinsic susceptibility to gonococcal infection based on blood group differences can be made. Analysis of the results by blood group or ethnic group was not attempted in the present study.

Another possibility is that our results, and those of Willcox *et al.* (1977), do not indicate that specific host factors are involved in individual susceptibility but rather reflect that the more frequent acquisition of both organisms is related to sexual behaviour. It has been suggested (World Health Organisation, 1978) that more frequent changes of sexual partner may have contributed to the increase in gonorrhoea found in many countries in recent years. Assuming that an intimate activity, such as kissing, is a common prelude to sexual intercourse, more frequent changes of partner might increase the chance of acquiring meningococci in the pharynx.

If the population examined in this study includes a group whose carriage of neisseriae merely reflects the association of intimate behaviour with the exchange of flora, then it might be worthwhile to investigate the carriage of other 'marker' organisms, for example, *Streptococcus pyogenes* and *Haemophilus influenzae*. If oropharyngeal carriage of these organisms indicates a similar correlation with genital gonorrhoea then the postulated host susceptibility factor for neisseriae is unlikely.

We thank Professor J. G. Collee for constructive criticism and helpful advice in the preparation of this paper.

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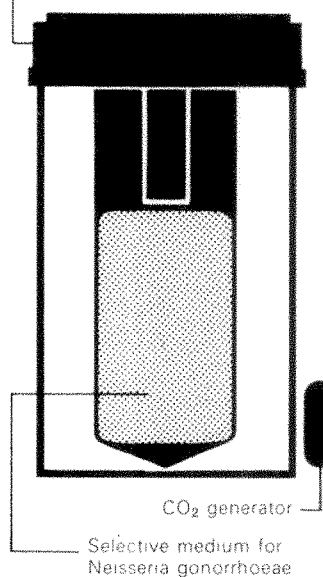
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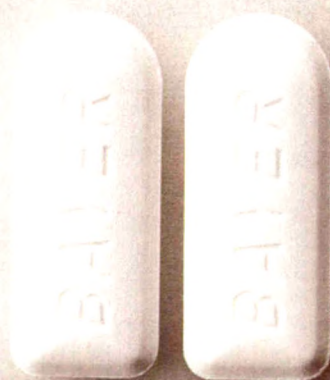
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Mezlocillin in the treatment of gonorrhoea

A. LASSUS AND O.-V. RENKONEN

From the Department of Dermatology and Venereology, University Central Hospital, Helsinki, and the Department of Serology and Bacteriology, University of Helsinki, Finland

SUMMARY A single, intramuscular dose of 2 g of mezlocillin was used for the treatment of 158 patients with gonorrhoea. In only three out of 144 patients with urogenital gonorrhoea who were re-examined did the treatment fail. Of 12 patients with pharyngeal gonorrhoea who were examined after treatment 10 were treated successfully with the same dose. The sensitivities of the gonococci to mezlocillin and penicillin G correlated well, although mezlocillin seemed to be active at lower concentrations on both highly sensitive and less sensitive isolates.

Introduction

Mezlocillin is a newly developed, semi-synthetic penicillin for parenteral administration. *In vitro* it has an antibacterial activity encompassing those of the cephalosporins, cephalothin and cephazolin, and the penicillins, ampicillin, carbenicillin, and tircacillin. Its antibacterial spectrum also includes, therefore, *Neisseria gonorrhoeae*. *In vivo* mezlocillin is active against certain Gram-negative bacteria, such as *Escherichia coli*, *Klebsiella*, *Proteus*, spp., *Pseudomonas aeruginosa*, and *Haemophilus influenzae*. It is also active against enterococci and other Gram-positive bacteria which do not produce β -lactamase (Metzger, 1976). So far the effect of mezlocillin in the treatment of gonorrhoea has not been reported.

In the present study, 2 g mezlocillin was used in the treatment of gonorrhoea in men and women.

Patients and methods

All the 158 patients studied (101 men and 57 women) were diagnosed and treated at the outpatient department for venereal diseases at the University Central Hospital, Helsinki, between September 1977 and February 1978.

Specimens for direct microscopy and culture were taken from the urethra in male patients and from the urethra, cervix, and rectal mucosa in female patients before treatment and at each follow-up visit. In addition, specimens for culture were taken from the

pharynx of 30 male patients and 25 female patients who had had orogenital contact. The male patients were seen once, seven to 14 days after treatment, and the female patients twice, seven to 14 and 14 to 21 days after treatment. The specimens for culture were transported in Stuart's medium to the department of serology and bacteriology, at the University of Helsinki, and plated within eight hours of being collected. In the laboratory the specimens were cultured on two Thayer-Martin media, one of which also contained trimethoprim. Although treatment was given on the basis of positive smear results, only the cases confirmed by culture (including fermentation reactions) were included in the assessment. Sensitivity tests to penicillin G and mezlocillin were carried out by the plate-dilution method described by Reyn *et al.* (1958).

A freshly prepared solution of 2 g of mezlocillin in 10 ml of saline was given as an intramuscular injection to both male and female patients.

Results

The results of the sensitivity tests to mezlocillin and penicillin G are illustrated in the Figure. A clear correlation is evident between the sensitivity of the gonococcal strains to the two antibiotics. Mezlocillin was more active, however, than penicillin on both highly sensitive and less sensitive isolates. All three patients who were considered true treatment failures harboured gonococci which had a decreased sensitivity to both mezlocillin and penicillin G.

Four male patients still had positive culture results from urethral specimens when re-examined (Table 1). One of them admitted to possible reinfection and was treated with a further dose of 2 g mezlocillin. He had

Address for reprints: A. Lassus, Department of Dermatology and Venereology, University Central Hospital, Snellmanink 14, Helsinki 17, Finland

Received for publication 7 June 1978

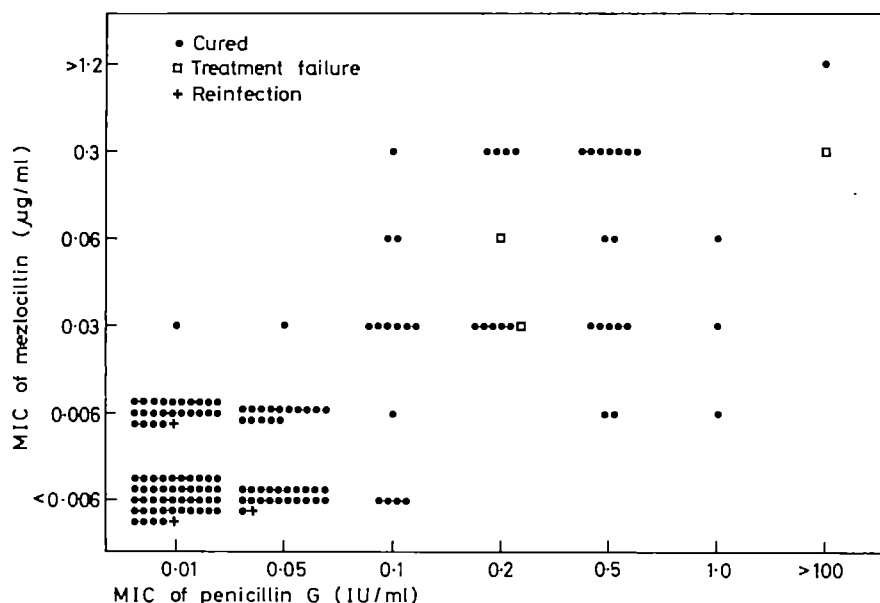


Figure Sensitivity of gonococci to mezlocillin and penicillin G for 158 patients with gonorrhoea. (Three strains were lost in subculture)

Table 1 Results of treatment of urogenital gonorrhoea with mezlocillin

Patients	Followed up		With positive cultures after treatment	
	Treated	No. %	No.	% of those followed up
Male	101	92 91	4	4
Female	57	52 91	2	4
Total	158	144 91	6	4

a negative culture result when re-examined one week later. In this case the gonococci were initially very sensitive to mezlocillin (MIC=0.006 µg/ml). In the remaining three cases the gonococci showed less sensitivity to mezlocillin (MIC=0.03, 0.06, and 0.3 µg/ml respectively). The latter strain was of the β -lactamase-producing type. These three patients were considered to be treatment failures and were all retreated with 2.4 g of spectinomycin, after which they were cured.

Two female patients had a positive culture result from the cervix when re-examined one at eight days and one at 13 days after treatment; both had gonococci which were sensitive to mezlocillin (MIC=0.006 µg/ml) and both admitted to possible reinfection. Both patients were cured after further treatment with 2 g of mezlocillin. A third female patient had gonococci of the β -lactamase-producing type with an MIC for mezlocillin >1.2 µg/ml. She was subsequently treated with spectinomycin (4 g)

despite a negative culture result seven days after she had been treated with mezlocillin. She also had negative culture results when subsequently examined on three occasions.

Seven male patients and seven females had positive gonococcal culture results (supported by sugar fermentation findings) from pharyngeal swabs before treatment. Two of the male patients still had a positive pharyngeal culture result when re-examined one week after mezlocillin treatment (Table 2). All the remaining 10 patients who were re-examined had negative pharyngeal culture results one, two, and three weeks after treatment with mezlocillin.

Mezlocillin was well tolerated in all cases, and none of the patients complained of pain at the injection site.

Discussion

The present study showed a cure rate of about 98% for cases of uncomplicated urogenital gonorrhoea with 2 g of mezlocillin. The cure rate was somewhat higher than the 95-96% achieved with the routine treatment (2.4 megaunits procaine penicillin plus 1 g probenecid) used at the same clinic. The same dosage of mezlocillin was used in both male and female patients, but the only true relapses occurred in male patients.

The high cure rate in the cases of pharyngeal gonorrhoea was of special interest. Single doses of ampicillin or pivampicillin combined with

Table 2 Results of treatment of pharyngeal gonorrhoea with mezlocillin

Patients	Followed up			With positive cultures after treatment	
	Treated	No.	%	No.	% of those followed up
Male	7	7	100	2	29
Female	7	5	71	0	0
Total	14	12	86	2	17

probenecid are ineffective in more than 50% of cases of pharyngeal gonorrhoea (Bro-Jørgensen and Jensen, 1973). The same seems to be true for high intramuscular, single doses of penicillin G. Mezlocillin seems worth trying for the treatment of larger series of patients with pharyngeal gonorrhoea.

The sensitivity of the gonococci to mezlocillin and penicillin G correlated well, although mezlocillin seemed to be active at lower concentrations on both highly sensitive and less sensitive isolates. Cross-resistance could be expected as both antibiotics are penicillins.

Thus a single dose of 2 g of mezlocillin is highly effective in the treatment of uncomplicated urogenital gonorrhoea and has a promising effect on cases of pharyngeal gonorrhoea.

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Growth and effect of chlamydiae in human and bovine oviduct organ cultures

G. R. HUTCHINSON, D. TAYLOR-ROBINSON, AND R. R. DOURMASHKIN

From the Division of Communicable Diseases and Electron Microscopy Section, Clinical Research Centre, Harrow, Middlesex

SUMMARY Organ cultures of 10 Fallopian tubes were inoculated with a genital strain of *Chlamydia trachomatis* and seven were infected. Infection was enhanced by centrifuging the organisms on to the tissues, larger numbers of organisms being reisolated from the tissues after this procedure. There was evidence of chlamydial multiplication because the number of organisms which were recovered from the tissues three to five days after inoculation had increased. Recovery was rare, however, after the sixth day, thus suggesting a self-limiting infection. Organ cultures of two bovine oviducts were infected with the bovine abortion strain of *Chlamydia psittaci*, but in these experiments centrifugation of the inocula did not enhance infection. The organisms were found in both the tissue and medium of cultures up to 18 days after inoculation and in much greater numbers than in the *C. trachomatis*-infected Fallopian cultures. Chlamydial infection was not entirely host-tissue specific, because *C. trachomatis* organisms were isolated from bovine oviduct cultures. Inclusions, however, were not detected histologically or electron microscopically in the epithelium of *C. trachomatis*-infected cultures, but they were detected by these means in *C. psittaci*-infected bovine cultures. All the elements of the chlamydial growth cycle were seen by electron microscopy, organisms being found in ciliated and possibly non-ciliated cells, and shedding of some infected epithelial cells was observed. No evidence of extensive epithelial cell damage was observed, however, and no loss of ciliary activity was detected in cultures infected with either *C. trachomatis* or *C. psittaci* when compared with uninoculated cultures. Thus acute salpingitis, when caused by chlamydial infection, is probably immunologically mediated.

Introduction

Chlamydia trachomatis is a cause of several diseases in human subjects, including trachoma and inclusion conjunctivitis and non-specific urethritis in men. In women, however, the association of chlamydiae with genital disease is less clear. Although chlamydiae can be isolated from women with cervicitis, and may cause the disease in some (Oriel *et al.*, 1974; Rees *et al.*, 1977), they can also be isolated from apparently healthy women (Oriel *et al.*, 1974). The association between chlamydiae in the cervix and acute pelvic inflammatory disease is more obscure, but the direct isolation of chlamydiae from Fallopian tubes of patients with acute salpingitis (Eilard *et al.*, 1976; Mårdh *et al.*, 1977) suggests that the organisms are

pathogenic in the upper genital tract. To substantiate this, further isolation and serological studies are required. In addition, however, studies of Fallopian tube organ cultures experimentally infected with chlamydiae might prove valuable. This system has already been useful in studying the pathogenicity of other micro-organisms, including mycoplasmas (Taylor-Robinson and Carney, 1974; Stalheim *et al.*, 1976; Mårdh *et al.*, 1976) and *Neisseria gonorrhoeae* (McGee *et al.*, 1976), and the mechanisms underlying the gonococcal disease process (Johnson *et al.*, 1977).

We have investigated the effect of *C. trachomatis* on human Fallopian tube organ cultures and of *Chlamydia psittaci* on bovine oviduct organ cultures.

Materials and methods

MEDIA

Before inoculation, organ cultures were maintained in Earle's Minimal Essential Medium with

Address for reprints: Dr D. Taylor-Robinson, Division of Communicable Diseases, Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ

Received for publication 6 October 1978

L-glutamine (Gibco Biocult) containing 50 µg/ml streptomycin, 100 µg/ml vancomycin, 50 units/ml nystatin, 0.025 mol/l HEPES buffer and NaHCO₃ to adjust the pH to 7.4.

After inoculation of chlamydiae, organ cultures were maintained in the medium described above supplemented with 10% fetal calf serum and 5% glucose.

TISSUE AND PREPARATION

Human Fallopian tube tissue was obtained from premenopausal women undergoing abdominal hysterectomy at Northwick Park Hospital. Bovine oviducts were obtained from animals at slaughter at the Institute for Research on Animal Diseases (Compton) and were placed in maintenance medium for transport to the laboratory. Organ cultures were prepared within one hour of removal of human Fallopian tubes and within two hours of removal of bovine oviducts. The tubes were placed in maintenance medium and the procedures previously described for organ culture preparation (McGee *et al.*, 1976) were used. Two to three pieces of tissue, each about 0.75 cm², were placed in 5-cm plastic Petri dishes containing 2 ml of maintenance medium. The cultures were incubated at 37°C in an airtight, plastic box gassed with 5% CO₂ in air. Immediately before inoculation the maintenance medium was replaced by growth medium and one hour after inoculation this was removed and replaced by fresh growth medium.

INOCULATION OF ORGAN CULTURES

The inocula comprised a strain of *C. trachomatis* (78a) isolated from a patient with nongonococcal urethritis, which had been passed twice in irradiated McCoy cells, and the bovine abortion strain of *C. psittaci* passed three times in McCoy cells. Each dish contained 0.2 ml of chlamydial inoculum and 1.8 ml of growth medium, which produced a final concentration of 10⁴-10⁵ inclusion-forming units per ml of medium. In some experiments, the pieces of tissue were placed mucosal-side uppermost in 1-cm diameter, flat-bottomed tubes, and the inoculum in 2 ml of medium was centrifuged on to the tissue at 2800 × g for one hour.

Organ cultures used as controls were inoculated with growth medium alone and were treated in all procedures in the same way as infected cultures.

ISOLATION OF CHLAMYDIAE

A piece of tissue, about 2 mm², was cut from a larger piece each day. The tissue was homogenised in a Ten-Broeck grinder in 0.5 ml of fresh growth medium, agitated with glass beads on a Vortex mixer for 30 seconds, and then centrifuged at 1000 r/min for five

minutes to remove cell debris. The supernatant fluid was mixed with an equal volume of sucrose-phosphate buffer and stored in liquid nitrogen. In addition, 0.5 ml of medium was removed from a dish and similarly stored. In four of 10 experiments using *C. trachomatis*-infected tubes, tissue samples were homogenised in 0.5 ml of the medium from the same dish and no separate medium sample was taken. The stored samples were later inoculated on to irradiated McCoy cells on coverslips for isolation of chlamydiae (Prentice *et al.*, 1976). After incubation at 36°C for 48 hours, the cells were fixed in methanol, stained with Giemsa, mounted, and examined by light and darkground microscopy to detect inclusions. An accurate assessment of the number of inclusions was not possible since the cell monolayers were often partially obscured by cell debris.

ASSESSMENT OF CILIARY ACTIVITY

The ciliary activity of tissue pieces was evaluated by means of an inverted microscope (×100 magnification) as described previously (McGee *et al.*, 1976). Dishes inoculated with chlamydiae and uninoculated controls were coded before observation to avoid bias.

HISTOLOGY

Specimens were fixed in formol saline, processed by routine histological methods, and stained with haematoxylin and eosin, Giemsa, or Macchiavello's stain.

ELECTRON MICROSCOPY

Specimens were fixed in 3% glutaraldehyde in 0.1 mol/l cacodylate buffer (pH 7.4), rinsed in 0.1 mol/l cacodylate buffer with 5% sucrose, and then post-fixed in 1% OsO₄ in 0.1 mol/l cacodylate buffer. They were dehydrated in acetone and epoxypropane and then embedded in Spurr resin. Sections were cut and stained with uranyl acetate and lead citrate. Electron microscopy was carried out in a Philips EM 300.

Results

INFECTION OF ORGAN CULTURES

C. trachomatis in Fallopian tube organ cultures

In 10 experiments with different tubes, no organisms were reisolated from the tissue of cultures two hours and one day after inoculation and none, or very few (less than 10 inclusions/coverslip), from the medium. Organisms were isolated, however, from the tissue and, where tested, occasionally from the medium on the second day and subsequently up to day 11, when the experiments were terminated. In all, seven tubes were successfully infected, chlamydiae being

reisolated from the tissues in all these cases and from the medium in one experiment where it was tested separately.

Effect of centrifuging the inoculum

In four experiments, the result of centrifuging the inoculum on to the tissue was compared with that of not doing so. As shown in Table 1, all tissues were infected following centrifugation and also when centrifugation was not undertaken. After centrifugation, however, the number of organisms in the tissues, as judged by the number of inclusions detected in McCoy cells, was at least fivefold greater than without centrifugation.

Table 1 *Effect of centrifugation on infection of Fallopian tube organ cultures by C. trachomatis organisms*

Experiment no.	Total no. of organisms* reisolated from tissue and medium	
	Inoculum centrifuged	Inoculum not centrifuged
1	63	3
2	224	48
3	168	23
4	531	111

* As assessed by inclusions in Giemsa-stained x-irradiated McCoy cells

Duration of infection

As shown in Table 2, where the results of experiments with centrifuged inocula are presented, the number of chlamydiae detected in the tissues was usually maximal three to five days after inoculation and then decreased thereafter. Apart from one experiment, organisms were not detected after the sixth day whether or not the inocula had been applied by centrifugation. These results suggested that the chlamydial infection was self-limiting in most cases.

Table 2 *C. trachomatis organisms in Fallopian tube organ cultures on different days after inoculation*

Experiment no.	No. of chlamydial organisms detected in tissues on indicated days after centrifugation of inocula							
	0	1	2	3	4	5	6	7
1		ND	ND		45	18	ND	ND
2		ND		20	180	24	ND	ND
3		ND	18	57	90	3	ND	ND
4		ND	ND	159	228	21	49	5
5		ND	ND	5	2	5	1	ND

*Chlamydiae detected up to day 11 in this experiment
ND = not detected

C. psittaci in bovine oviduct organ cultures

In two experiments with different tubes, no organisms were isolated one day after inoculation.

Two to three days later, however, they were recovered in equal numbers from both the tissue and the medium each day up to day 14 or 18 when the experiments were terminated. Maximum numbers of organisms (approximately 2×10^4 inclusions/coverslip) were detected 10 to 12 days after inoculation. This was about 100-fold more organisms than detected in *C. trachomatis*-infected cultures. Centrifugation of the inoculum did not seem to enhance the extent or duration of infection by *C. psittaci*.

Specificity of infection

In one experiment, *C. trachomatis* organisms did not infect a rabbit oviduct. Infection was not limited, however, to human tissue because these organisms infected bovine oviduct organ cultures in two experiments, in one of which inoculation was not assisted by centrifugation. The organisms were detected only in the tissues and not beyond the fifth day.

CILIARY ACTIVITY

Uninoculated organ cultures of human and bovine oviduct usually retained some ciliary activity for two to three weeks, although ciliary activity of all pieces gradually decreased over this time. Centrifugation seemed to have no effect on ciliary activity.

C. trachomatis in Fallopian tube organ cultures

Ciliary activity was assessed in six experiments. Activity was reduced by about 50% in two of three experiments in which there was no evidence of chlamydial infection. In the three experiments in which infection occurred, however, no loss of ciliary activity was detected compared with that of the uninoculated control cultures.

C. psittaci and C. trachomatis in bovine oviduct organ cultures

In two experiments of 14 and 18 days' duration, despite good evidence of infection by *C. psittaci*, no significant loss of ciliary activity occurred compared with that of uninoculated control cultures. The results of one experiment are presented in Fig. 1; the apparently greater loss of ciliary activity in *C. psittaci*-infected cultures noted early after infection was not statistically significant (day three, $P > 0.05$; Student's *t* test with 2 d.f.). Furthermore, *C. trachomatis* appeared to have no effect.

HISTOLOGICAL APPEARANCE OF TISSUES

Chlamydial inclusions in cells were sought on days one, five, seven, and 12 after inoculation of oviduct organ cultures. Inclusions were not seen in sections from cultures inoculated with *C. trachomatis*. In

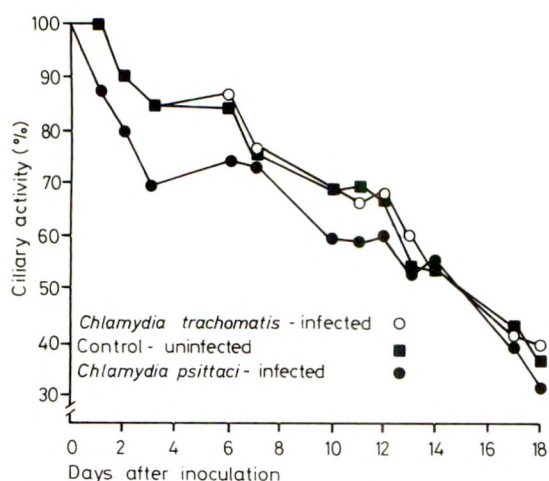


Fig. 1 Ciliary activity of bovine oviduct organ culture after infection by the bovine abortion strain of *C. psittaci* or *C. trachomatis*

sections from bovine cultures inoculated with *C. psittaci*, however, inclusions were detected on day five after inoculation and were easily seen on days seven (Fig. 2) and 12. The inclusions were observed in

the cytoplasm of cells near the mucosal surface. Although they were seen in cells which did not appear to possess cilia it was not possible to determine whether or not these cells had been ciliated originally. The inclusions were distinct, stained dark-blue by Giemsa, and contained particles.

Despite the presence of observable inclusions, at least in the bovine tissues, there was no obvious change in the architecture of the mucosal epithelium of the bovine-infected or human-infected tissues compared with that of the uninoculated controls. Most of the epithelium was ciliated after 12 days in culture, although some sub-epithelial degeneration occurred in both infected and control cultures.

ELECTRON MICROSCOPY

Chlamydial inclusions in cells could not be detected in sections of Fallopian tube organ cultures inoculated with *C. trachomatis*. Observations were made on sections of *C. psittaci*-infected bovine oviduct cultures four and seven days after inoculation. Epithelial cells which were seen tended to possess only microvilli—and were, therefore, apparently non-ciliated—and to stain more densely

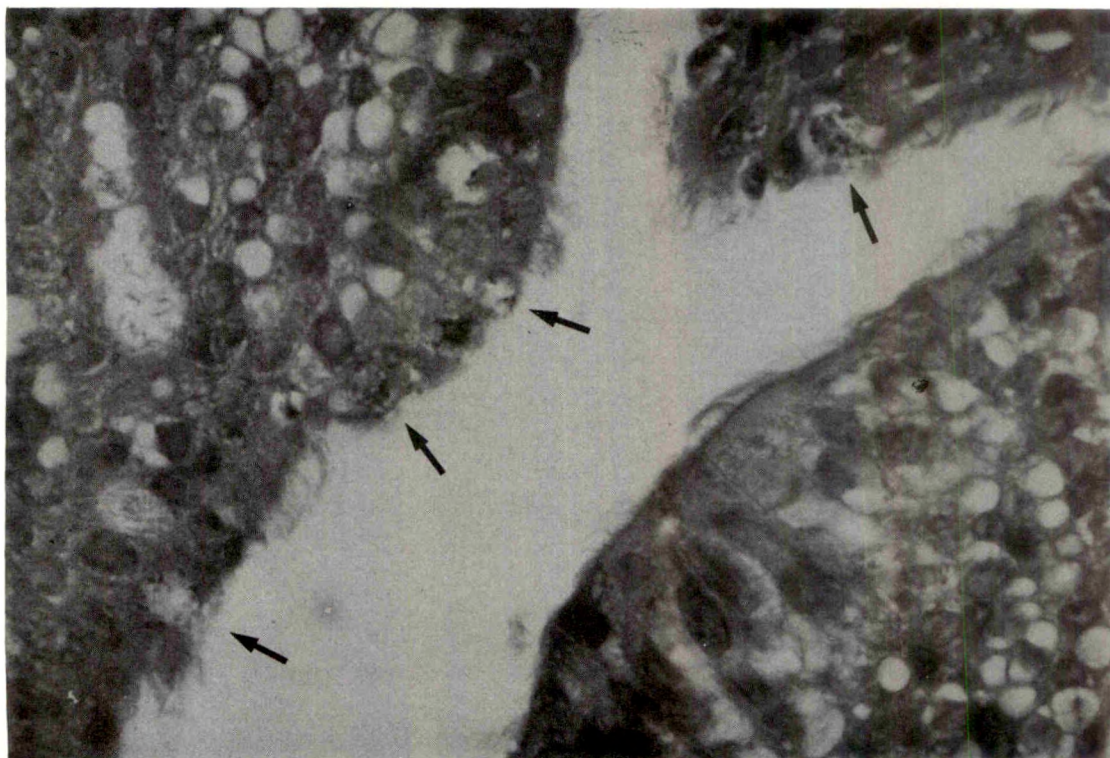


Fig. 2 Histological section of *C. psittaci*-infected bovine oviduct organ culture. Inclusions (arrowed) in epithelial cells seven days after inoculation. Giemsa $\times 810$

than cells which had both microvilli and cilia (Fig. 3). Inclusions were not seen four days after inoculation but they were seen seven days after. They were observed occasionally in epithelial cells which were obviously ciliated (Fig. 4) but more frequently in cells bearing microvilli only. Some of the latter were probably mucus-secreting, non-ciliated cells because they stained densely. It was impossible to know, however, whether or not some of these cells had been

ciliated originally but had lost their cilia as a consequence of infection. Some epithelial cells showed evidence of early infection with reticulate bodies only in the cytoplasm (Fig. 5a), while in others infection was more advanced, the inclusions containing reticulate and elementary bodies (Figs. 3, 4, and 5b). The cytoplasm and nucleus appeared normal until the late stages of cell infection when the nucleus was sometimes compressed by an inclusion

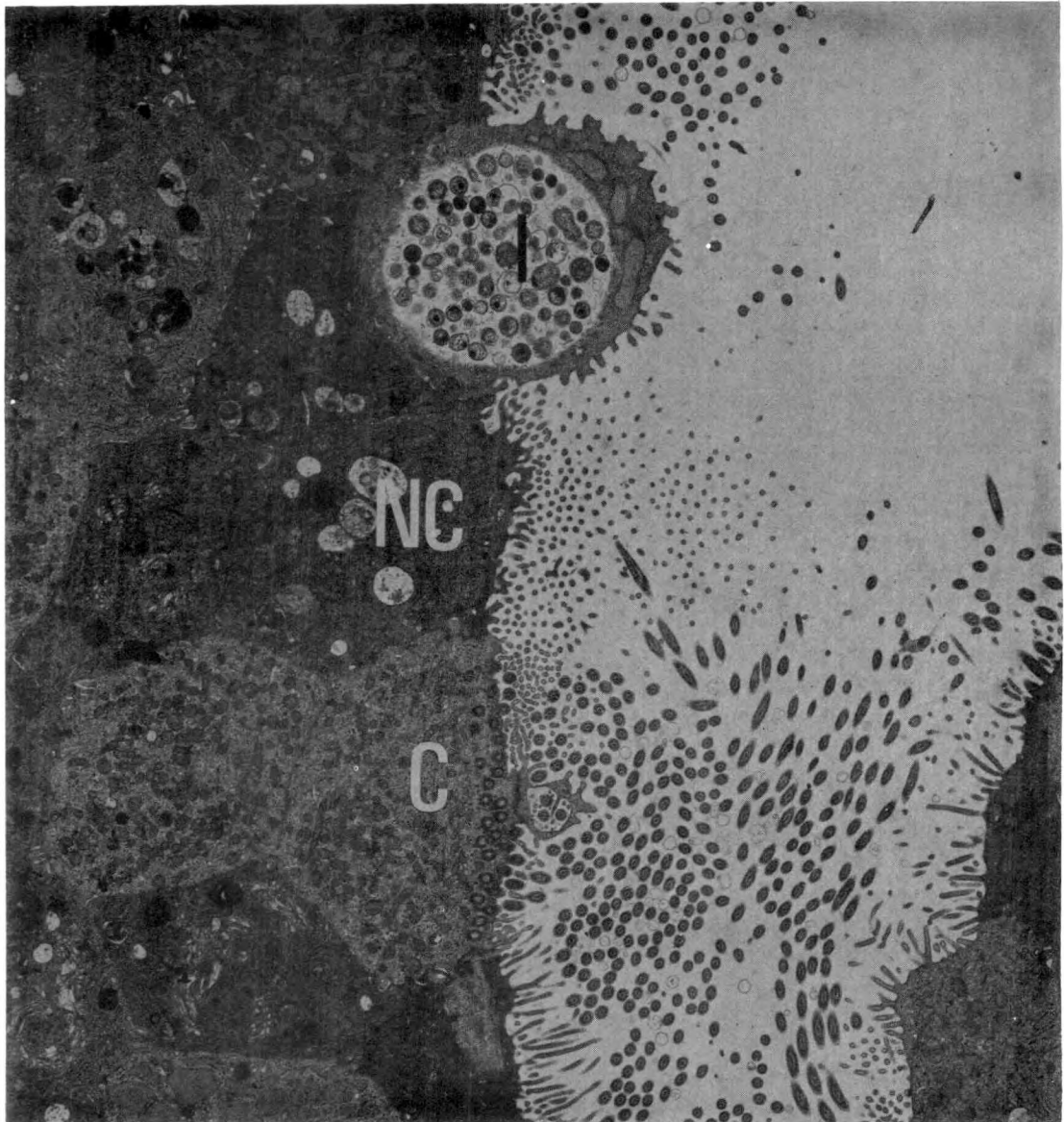


Fig. 3 Electron micrograph of *C. psittaci*-infected bovine oviduct organ culture seven days after inoculation ($\times 5800$). I—inclusion; C—ciliated epithelial cell; NC—non-ciliated epithelial cell

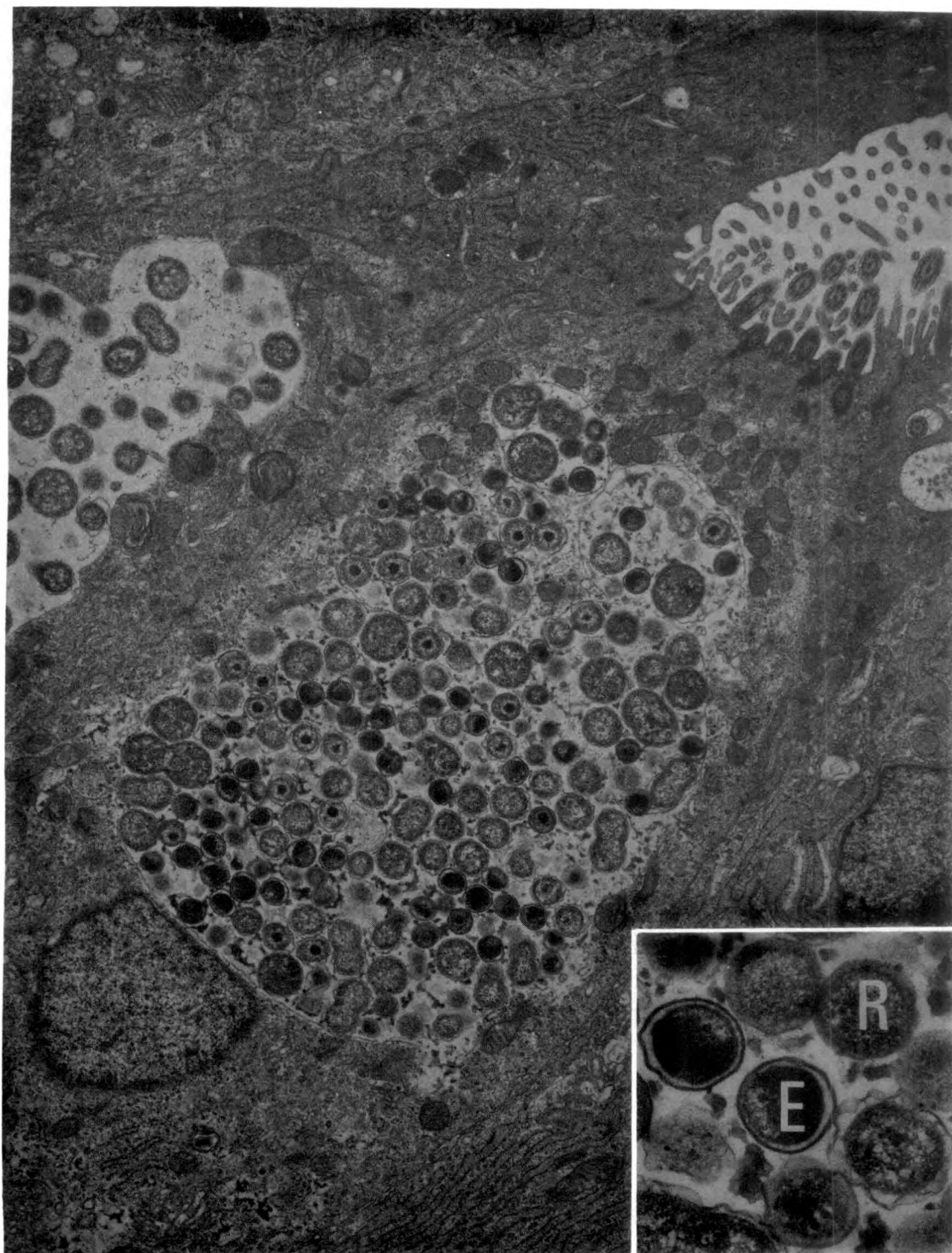
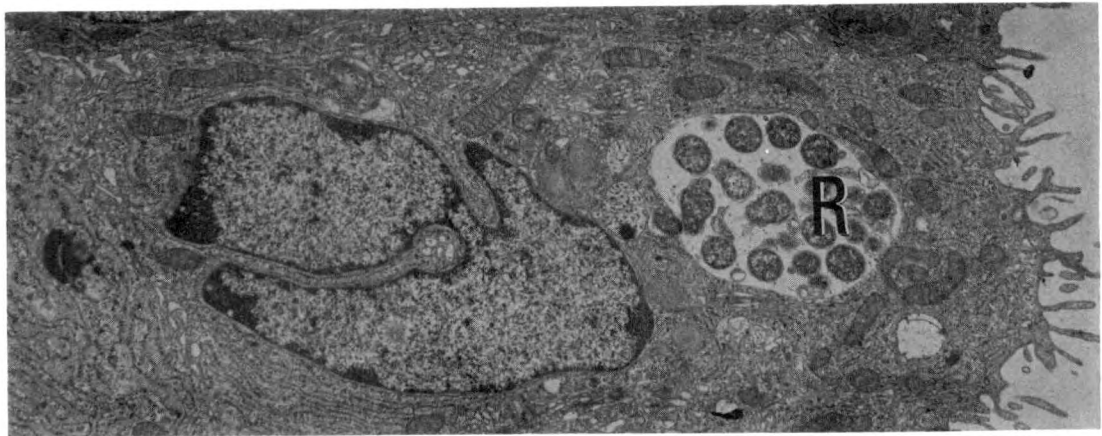
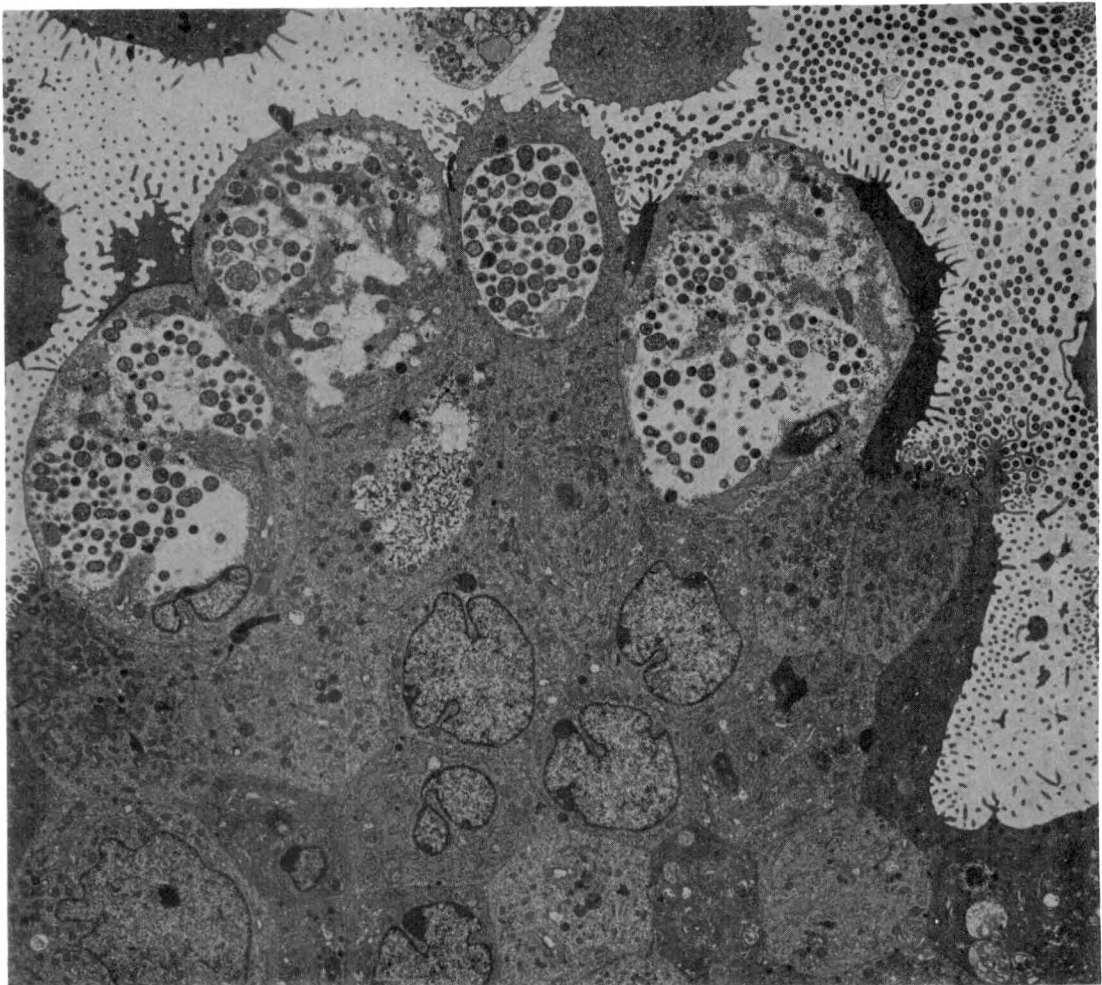


Fig. 4 (and inset) Electron micrograph of *C. psittaci*-infected bovine oviduct organ culture seven days after inoculation. Inclusion in ciliated epithelial cell ($\times 11\ 600$). Inset: E—elementary body, R—reticulate body ($\times 42\ 640$)



(a)



(b)

Fig. 5 Electron micrographs of *C. psittaci*-infected bovine oviduct organ culture seven days after inoculation: (a) Inclusion-containing reticulate bodies (R) ($\times 6400$); (b) Inclusion-containing cells being extruded from mucosal surface ($\times 3680$)

occupying almost the whole of the cytoplasm. At this stage, a cell containing many chlamydial particles was seen occasionally to be in the process of being extruded from the mucosal surface (Figs. 3 and 5b). Processing the tissues for electron microscopy may have contributed to the failure to see extracellular chlamydial particles.

Discussion

The results of our attempts to infect human and bovine oviduct mucosae with chlamydiae under experimental conditions show that the oviduct tissues are susceptible to infection. Multiplication of *C. trachomatis* organisms occurred in seven of 10 Fallopian tubes in organ culture. Likewise, replication of organisms of the bovine abortion strain of *C. psittaci* occurred in bovine oviducts, and inclusions produced by these organisms were observed both by light and electron microscopy. Furthermore, replication of the *C. psittaci* strain without centrifugation of the inocula on to the tissues was not difficult to demonstrate, but the small numbers of inclusion-forming units produced by *C. trachomatis* in Fallopian tube cultures were increased by centrifugation of the inocula. The phenomenon of intracellular inclusions increasing in number as a result of inoculum centrifugation has been noted previously for *C. trachomatis* in cell cultures (Darougar *et al.*, 1974) and, of course, can have no parallel in the *in-vivo* clinical situation, where even small numbers of chlamydial organisms may be able to set up an infection. The apparent self-limiting infection produced by *C. trachomatis* in the organ cultures may be accounted for by the inability of a few liberated chlamydial particles to reinfect without further centrifugation, coupled with epithelial cells which become less susceptible to infection as the organ cultures age. The more prolonged infection by *C. psittaci* is probably due to liberation of large numbers of organisms into the culture medium and their ability to infect without centrifugation.

The minimal infection of Fallopian tube organ cultures, as indicated by the small number of inclusions which was detected, clearly accounts for the failure to find histological and electron microscopical changes, particularly when only small fragments of tissue were examined. The more extensive infection of bovine oviduct cultures, as indicated by the large number of *C. psittaci* organisms detected in the medium and tissue of the cultures, is in keeping with the ability to observe some histological and electron microscopical changes. These changes, in which all the elements of the chlamydial growth cycle were seen, were similar to those which have been observed in experimentally

infected cell cultures (Manire, 1977) and in cervical epithelium naturally infected by *C. trachomatis* (Swanson *et al.*, 1975). Infection of ciliated epithelial cells and shedding of infected cells from the epithelial surface was evident. Despite this, however, ciliary activity was not seen to be affected, even though a quantitative method of assessment was employed (McGee *et al.*, 1976).

It is known that *C. trachomatis* may infect the tissues of the female genital tract without causing pathological or cytological changes, because chlamydiae have been isolated from women who show no signs of disease (Oriel *et al.*, 1974), but there is also some evidence that chlamydiae may cause cervicitis (Rees *et al.*, 1977) and salpingitis (Eilard *et al.*, 1976; Mårdh *et al.*, 1977). Our observations on experimental infection of oviduct cultures must raise the question of their relevance to such natural infections. There is clearly a dilemma. It is not possible to put forward the notion that the minimal changes produced in the organ cultures suggest that chlamydiae are likely to cause only minimal changes *in vivo*, because the organ culture system is unlikely to represent completely the *in-vivo* situation. Furthermore, without unquestionably knowing that *C. trachomatis* causes acute salpingitis it is difficult to use the model as a means of explaining the purported pathological changes. It is possible that the clinical disease occurs as a result of a combined infection with chlamydiae and other micro-organisms or, alternatively, that tissue which has already been damaged by infection with another micro-organism provides a suitable environment for the growth of chlamydiae which take no active part in producing the disease. If, however, we assume that chlamydiae alone are involved in causing acute salpingitis, since recent evidence points further in that direction (Treharne *et al.*, 1979), then it seems likely that the inflammatory changes—rather than being a direct effect of the organisms on the tissues, as may occur in gonococcal infections (Carney and Taylor-Robinson, 1973)—represent an immunological response of the host to chlamydial infection. This proposal would be compatible with the failure of chlamydiae to affect appreciably the tissues in organ culture where they are separated from the immunological systems of the host and would be in keeping with the known importance of immune factors in causing severe chlamydial-stimulated disease in other anatomical areas such as the eye.

We thank Mr A. M. Fisher and Mr H. Gordon (Northwick Park Hospital) for providing Fallopian tubes and Dr R. N. Gourlay (Institute for Research on Animal Diseases, Compton) for helping us to

obtain bovine oviducts. We also thank Mr C. Sowter and Mr J. Clark, for histopathology and photomicrography respectively, and Mr D. Shah for help with electron microscopy.

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Chlamydia trachomatis in acute salpingitis

J. PAAVONEN,* P. SAIKKU,† E. VESTERINEN,* AND K. AHO‡

From the *I-II Departments of Obstetrics and Gynaecology, Helsinki University Central Hospital;

†Department of Virology, University of Helsinki; and the ‡Central Public Health Laboratory, Helsinki, Finland

SUMMARY In a study to evaluate the possible role of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in acute salpingitis, 26% of 106 patients with severe symptoms had positive culture results for *C. trachomatis*; 43% of the 72 patients from whom paired sera were obtained had either positive culture results for or seroconversion in the single antigen immunofluorescence test to *C. trachomatis*. Twenty-six per cent of patients harboured *N. gonorrhoeae* and 14% had gonococcal complement-fixing antibody titres ≥ 8 . Intrauterine devices were used by 48% of patients, no difference being found in the frequency of use between patients harbouring *C. trachomatis* or *N. gonorrhoeae*. The possible role of *C. trachomatis* should be considered in the treatment of acute salpingitis.

Introduction

Acute salpingitis is a common disease and seems to be increasing. In 99% of such cases the infection ascends from the lower genital tract, usually the cervix (Falk, 1946, 1965; Sweet, 1977). The remaining cases are caused by tuberculous salpingitis or by infections which spread from nearby pelvic structures, such as the appendix. Despite the high incidence (Widholm and Kallio, 1965; Weström and Mårdh, 1977) and morbidity (Weström, 1975) of acute salpingitis, the cause of the tubal infection remains uncertain in a large proportion of cases. Acute salpingitis has been associated with *Neisseria gonorrhoeae* (Falk, 1965; Sweet, 1977; World Health Organisation, 1978), with *Mycoplasma hominis* and T-mycoplasma (Mårdh and Weström 1970), and with mixed aerobic and anaerobic bacterial pathogens (Chow *et al.*, 1975; Eschenbach *et al.*, 1975). A significant decrease in the percentage of cases of salpingitis attributed to *N. gonorrhoeae* has been noted, however, during the past decade in Scandinavia (Kallings and Moberg, 1977; Weström and Mårdh, 1977). Recently, *Chlamydia trachomatis* has attracted attention as an important infective agent (Schachter, 1978). A relationship between *C.*

trachomatis infection and acute salpingitis was suggested by Dunlop and his co-workers in 1966 (Dunlop *et al.*, 1966). More recently, Mårdh *et al.* (1977a) recovered *C. trachomatis*, during laparoscopy, from six out of 20 specimens from the Fallopian tubes of patients with acute salpingitis compared with zero out of 12 specimens from patients with no signs of genital infection.

The present work is a prospective study of the frequency of urogenital *C. trachomatis* and *N. gonorrhoeae* infections in patients admitted to hospital with acute salpingitis and evaluates the possible role of chlamydiae in this disease using antibody studies.

Material and methods

PATIENTS

The study population consisted of 106 women with acute pelvic symptoms who attended the outpatient clinics of the first and second departments of gynaecology and obstetrics at the Helsinki University Central Hospital from October 1977 to June 1978.

The clinical diagnosis of acute salpingitis was based on common criteria: pelvic pain of short duration, tender adnexal masses, increased erythrocyte sedimentation rate, and, usually, fever. All the patients were admitted to hospital because of the severe symptoms. Ten patients had received antimicrobial treatment during the two weeks before admission.

Address for reprints: Dr J. Paavonen, I Department of Obstetrics and Gynaecology, University Central Hospital Hartmaninkatu 2, SF-00290 Helsinki 29, Finland

Received for publication 19 September 1978

The mean age of the patients was 27.3 years and the range, 15-49 years. Fifty-one (48%) patients were fitted with an intrauterine device (IUD), 10 (9%) were taking oral contraceptives, and the remainder used other methods or none at all.

CULTURE TECHNIQUES AND SEROLOGICAL METHODS

Cervical and urethral specimens were cultured for *C. trachomatis* in irradiated McCoy cells (Paavonen *et al.*, 1978b). Serum antibodies were determined by the single antigen immunofluorescence test (IFAT) using *C. trachomatis* L2 (434 Bu) inclusions in dog kidney cells as antigen (Saikku and Paavonen, 1978). Acute and convalescent phase sera (obtained one to four weeks later) were obtained from 72 patients. Cervical and urethral specimens were cultured for *N. gonorrhoeae* by the conventional method (Lennette *et al.*, 1974). The gonococcal microcomplement fixation (CF) test was performed using a mixture of 20 recently isolated strains as the test antigen (Aho and Sievers, 1972). Statistical significance was evaluated by the χ^2 test with Yates's correction.

Results

Of the 106 patients with acute salpingitis, 27 (26%) harboured *C. trachomatis* in their urogenital tract, 12 in the cervix and urethra, 13 in the cervix alone, and two in the urethra alone. In paired sera 10 (46%) out of 22 chlamydia-positive patients and nine (18%) out of 50 chlamydia-negative patients showed significant ($\geq$ fourfold) change in IFAT titres (Figure). Thus, seroconversion occurred in 19 (26%) out of 72 patients with salpingitis from whom paired sera were obtained. The geometric mean titre (GMT) of titres ≥ 8 was 219 in chlamydia-positive patients and 73 in chlamydia-negative patients. The 10 patients in whom antimicrobial treatment was begun before culture was attempted were all chlamydia-negative. Five of them, however, showed significant change in IFAT titres in paired sera (Figure).

Of the 27 chlamydia-positive patients, eight (30%) also had gonorrhoea compared with 19 (24%) out of 79 chlamydia-negative patients (statistically not significant, $P > 0.1$). A total of 15 (14%) of the 106 patients had gonococcal CF titres of ≥ 8 . Of the 27 patients with positive culture results for *N. gonorrhoeae*, nine (33%) had titres of ≥ 8 compared with six (8%) of the 79 culture-negative patients (statistically significant, $P < 0.01$). The highest CF titre was 16, and even this occurred in only five patients, all of whom were culture-positive.

Of the 51 patients using an IUD, nine were chlamydia-positive, eight were *N. gonorrhoeae*-positive, and two harboured both organisms.

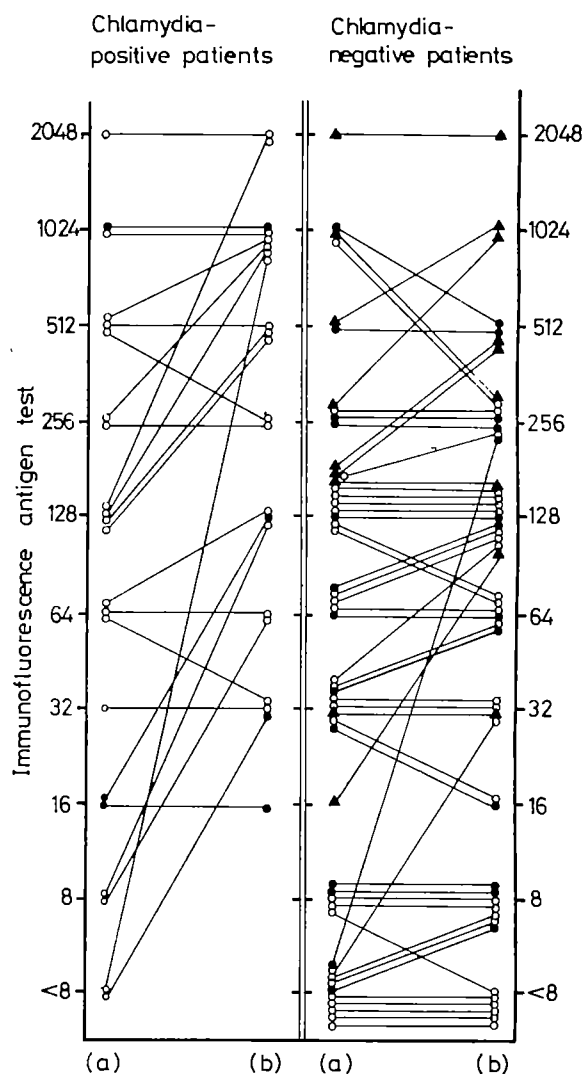


Figure Antibody titres in IFAT against *C. trachomatis* in paired sera from patients with acute salpingitis. (Acute phase (a) and convalescent phase (b) sera are connected with a line)

○ Negative gonococcal isolation; ● positive gonococcal isolation; ▲ antibiotic treatment begun before culture

Discussion

The results of the present study provide additional support for the concept that acute salpingitis may in many cases be due to infection with *C. trachomatis*; 26% of the patients harboured the organism, and 26% showed seroconversion in the single antigen immunofluorescence test. Seroconversion occurred more often ($\chi^2_1 = 4.60$; $P < 0.025$) among

chlamydia-positive patients than among chlamydia-negative patients. The average GMT in patients with acute salpingitis was high compared with that (GMT 27) in our earlier findings in women of fertile age (mean 29.5 years) representing hospital outpatients (Paavonen *et al.*, 1978b) or in asymptomatic but chlamydia-positive sexual partners of men with nongonococcal urethritis (GMT 80) (Saikku and Paavonen, 1978), thus suggesting systemic chlamydial infection.

Similarly, Eschenbach *et al.* (1975) noted rising micro-IF antibody titres to *C. trachomatis* in 24% of women with nongonococcal pelvic inflammatory disease. In addition, Lycke *et al.* (1976) demonstrated chlamydial antibodies in the haemolysis-in-gel test in sera from all seven chlamydia-positive patients with salpingitis and in four of 17 chlamydia-negative patients with salpingitis. Hamark *et al.* (1976) observed a significant rise in CF antibody titre in four patients with salpingitis from whom *C. trachomatis* was isolated from the cervix. In one of these women the organism was isolated from the Fallopian tubes as well.

The frequency of gonococcal infection was as low in our study as in the recent findings in Sweden (Danielsson *et al.*, 1975; Weström and Mårdh, 1977). The role of mycoplasmas as genital tract pathogens is under debate (Lancet, 1970; Mårdh and Weström, 1970; Eschenbach *et al.*, 1975; Vaughan-Jackson *et al.*, 1977; Paavonen *et al.*, 1978a). Furthermore, the isolation of anaerobes from the Fallopian tubes has not been successful (Weström and Mårdh, 1975; Mårdh *et al.*, 1977b).

It is still possible that the frequent finding of *C. trachomatis* in the urogenital tract reflects only the sexual mode of transmission of pathogens responsible for salpingitis or that chlamydial cervical infection allows other pathogens to ascend. Seroconversions and high antibody titres, however, provide evidence to the contrary; *C. trachomatis* seems to participate actively in this systemic infection. The fact that even chlamydia-negative patients had high IFAT titres (GMT 73) needs explaining. High titres may be due to a past chlamydial infection. Technical failure as well as recent treatment with antimicrobial agents might be responsible for the negative culture results in some cases.

In the present series the overall frequency with which IUDs were used was high (48%). This may be compared with the frequency of 29% among 202 sexually active partners of men with nongonococcal urethritis examined in the same clinic during the same period (Paavonen *et al.*, unpublished data). No difference was found between chlamydia-positive and *N. gonorrhoeae*-positive patients in the use of

IUDs. Other investigators have found a frequency rate of 24-81% in patients with salpingitis (Sweet, 1977). Weström *et al.* (1976) reported a significantly increased risk of acquiring acute salpingitis in patients using an IUD compared with non-users.

The late sequelae of salpingitis are well known: infertility, increased frequency of ectopic pregnancies, and chronic abdominal pain. The risk of spread of cervical infection to the Fallopian tubes must be considered in the treatment of cervical chlamydiae. The role of *C. trachomatis* in the aetiology of acute salpingitis necessitates a re-examination of present treatment guidelines. In addition, the isolation of *C. trachomatis* in many cases (30% in the present study) concomitantly with *N. gonorrhoeae* should influence the choice of treatment for gonococcal disease. A treatment regimen which is not totally effective against *C. trachomatis* may give rise to persistent, latent chlamydial infection which later requires further treatment for chronic salpingitis and its long-term sequelae. At the moment, the role of *N. gonorrhoeae* in the aetiology of salpingitis may well be overestimated.

This study was aided by a grant from the Finnish Cultural Foundation.

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HLA and sexually transmitted diseases in prostitutes

S. H. CHAN,* TULIP TAN,† ALI KAMARUDIN,† G. B. WEE,* AND V. S. RAJAN†

From the *Department of Microbiology, Faculty of Medicine, University of Singapore, and †Middle Road Hospital, Singapore

SUMMARY The HLA profile of 148 unrelated, Chinese prostitutes (56 with repeated gonococcal infection, 31 with syphilis, 31 with gonorrhoea and syphilis, and 30 with no evidence of infection) was compared with that of 238 unrelated, healthy, Chinese control subjects. The joint occurrence of AW19 B17 was observed in 25·8% of prostitutes with double infections compared with 6·7% of control subjects, while that of A11 B15, on the other hand, was associated with a resistance to syphilis and gonorrhoea. The latter profile was observed in 46·7% of prostitutes in business for more than two years who were resistant to disease, in 30% of prostitutes with an overall disease resistance, in 13·4% of control subjects, and in only 3·2% of prostitutes with combined syphilis and gonorrhoea. Because of the statistical uncertainty when multiple variables are being analysed these studies should be repeated in other groups of prostitutes of the same and different ethnic origins.

Introduction

Prostitutes are the major source of sexually transmitted diseases in Singapore (Khoo *et al.*, 1977). Some prostitutes have repeated infections while others are quite resistant to infection. While exposure to the causative organisms is an obvious explanation, another reason may be the differences in the immune response of the host to these organisms. The control of immune responses to synthetic antigens by immune response (IR) genes associated with the major histocompatibility locus has been clearly demonstrated in animals (Snell *et al.*, 1976). Recently, there has been evidence which suggests that IR genes associated with the major histocompatibility locus (HLA) may also exist in man (Blumenthal *et al.*, 1964; McMichael and McDevitt, 1977; Vries *et al.*, 1977). Thus, if the functions of immune response genes influence the susceptibility or resistance to venereal disease organisms in these prostitutes, these differences may be reflected in their HLA profiles. The results of HLA typing in prostitutes who are susceptible to and in those who are resistant to venereal diseases are reported in this paper.

Material and methods

STUDY POPULATION

A total of 148 unrelated, Chinese, female prostitutes were HLA-typed and the HLA profile compared with that of 238 unrelated, healthy, Chinese, control subjects. The control group consisted of normal, healthy babies born consecutively at a maternity hospital in Singapore over a period of nine months. Both parents were of Chinese ethnic origin, the same ethnic group as the prostitutes. All the prostitutes, who carry medical cards which are regularly checked, are seen at the Middle Road Hospital every two weeks for periods ranging from one to 20 years. Cervical and rectal swabs for culture of gonococci are taken at every visit, and serological tests for syphilis (Venereal Disease Research Laboratory and fluorescent treponemal antibody absorption tests) are performed every three months.

The prostitutes were subdivided into four groups; the ages and the time of exposure to infection of each group is given in Table 1.

Group 1

This group consisted of 56 patients with repeated gonococcal infections diagnosed by positive culture results. The average number of infections was 5·4 per year per patient, ranging from two to 24 infections per year, and the average interval before the first infection was 3·4 months.

Address for reprints: Dr S. H. Chan, WHO Immunology Training Centre, Faculty of Medicine, University of Singapore, Sepoy Lines, Singapore 3

Received for publication 15 August 1978

Table 1 *Details of age and exposure to infection of 148 Chinese prostitutes*

Patient group	Age (years)		Exposure to infection (years)*	
	Mean	Range	Mean	Range
1	35.8	18-53	5.5	1-17
2	39.3	23-55	10	1-20
3	37.3	24-56	6.7	1-20
4	32.5	19-52	4.3	1-20

* Estimated from the date each patient became a prostitute to the time of the study

Group 2

This group consisted of 31 patients with latent syphilis who had been treated and at the time of the study had no symptoms or signs of infection.

Group 3

This group consisted of 31 patients with combined infections. Serological tests for syphilis and repeated

cultures for gonococci both gave positive results. The average number of infections was 4.2 per year per patient, ranging from two to nine per year, and the average interval before the first infection was four months.

Group 4

This group consisted of 30 patients who had been exposed to gonorrhoea and syphilis for at least one year but were resistant to infection.

HLA TYPING

HLA typing was performed using the NIH (National Institutes of Health) lymphocyte micro-cytotoxicity method (National Institute of Allergy and Infectious Diseases, 1976-77). A total of 196 antisera defining 26 specificities were used. HLA typing sera were obtained from our own laboratory, the NIH serum bank, and direct from other laboratories. The sera defining A11, AW19, B15, and B17 are listed in Table 2.

Table 2 *Antisera used to define A11, AW19, B15, and B17*

Specificity	Antiserum		Contributor
HLA-11	Ross	2-70-3-04-07-01	NIH
	Davies	2-70-3-11-29-01	NIH
	Tellier	2-71-4-07-12-01	NIH
	S97/27		Singapore
	S98/95		Singapore
	S84/19		Singapore
	S101/56		Singapore
	AH184		Singapore
	AH44		Singapore
'AW19 complex'			
	AW30		
	Ortiz	2-50-5-08-13-01	NIH
	Abrams	2-73-2-02-10-01	NIH
	KC-OR	2-50-5-05-23-01	NIH
	Goggins	2-71-4-05-17-01	NIH
AW30 + AW31	Merle		Dausset
AW30 + AW31	S. Ouwenhand	1-46-5-02-12-01	NIH
AW30 + AW31	KR4282	1-46-5-02-12-01	NIH
AW32 + AW25	cc40	2-61-4-01-09-01	NIH
<AW33	Thompson	2-50-6-04-13-02	NIH
AW30 + 31 + 32 + 33 + A10	Rabatin	2-65-1-07-23-01	NIH
AW30 + 31 + 33 + A10	Ortiz	2-50-8-05-11-02	NIH
AW30 + 31 + 32 + 33	cc23.1	2-61-1-06-24-01	NIH
AW30 + 31 + 32 + 33	cc23.2	2-61-1-08-12-01	NIH
AW30 + 31 + 33 + A10	Quinones	2-57-0-05-05-01	NIH
AW32 + weak reaction	Castenda	2-50-1-09-09-01	NIH
B15	TH-WGJ		Thorsby
B15 + BW35	Tuit 12371	1-04-1-02-07-01	NIH
	Templer	2-83-2-12-01-01	NIH
	Staats	1-04-5-04-24-01	NIH
	W. Eickel	1-04-3-06-13-01	NIH
	G. W. Klomp	1-15-0-07-02-01	NIH
	S18/05R1		Singapore
	305K		Betuel
	S87/58		Singapore
	MMM	1-36-3-11-13-01	NIH
	118E		Betuel
	Sired		Joysey
B15 + weak reaction	W. H. Wickers	1-15-1-05-05-01	NIH
B15 + B17 + Sin 2 +	P. Duwenhand	1-04-2-12-20-01	NIH
B15 + B17 + Sin 2 +	cc61	2-61-4-10-30-01	NIH
B17	S. M. Abela		Hayes
	M. Daw		Hayes
	S102/16		Singapore
	S101/26		Singapore
	S18/26R1		Singapore

Results

The HLA antigen frequencies of the various groups of prostitutes and controls are shown in Table 3. The frequency of BW46 was significantly higher in the group with gonorrhoea and syphilis combined (group 3) whereas that of A2 was significantly lower in the resistant group (group 4) when compared with the controls. This difference, however, was not significant when corrected for the number of antigens typed.

Certain HLA-A-locus antigens occurred more frequently than expected with a specific B-locus antigen, a situation termed 'linkage disequilibrium'. The joint occurrences of AW19 B17 and A11 B15 in the various groups of prostitutes and in the controls are shown in Table 4. The joint occurrence of AW19 B17 was seen in eight (25.8%) out of 31 prostitutes with gonorrhoea and syphilis combined compared with 16 (6.7%) out of 238 controls ($\chi^2 = 12.29$; $P < 0.0005$; relative risk = 4.83). The joint

occurrence of A11 B15, on the other hand, was observed in nine (30%) out of 31 prostitutes resistant to disease compared with 32 (13.4%) out of 238 controls ($\chi^2 = 5.16$; $P < 0.025$) and one (3.2%) out of 31 prostitutes with combined infections ($\chi^2 = 5.84$; $P < 0.025$). Of the 30 prostitutes who were resistant to disease, 15 had been exposed for more than two years; of these 15, A11 B15 was observed in seven (46.7%) patients. The differences in the frequency of A11 B15 between this subgroup of prostitutes (exposed for more than two years and yet resistant to disease) and the controls ($\chi^2 = 11.9$; $P < 0.001$) and prostitutes with double infections ($P = 0.0008$) were even more pronounced.

Discussion

Caution must be exercised in interpreting the significance of a joint occurrence of antigens from two linked loci, since a large number of combinations (180 in this instance) are possible, and significant

Table 3 Frequencies of HLA in Chinese prostitutes and controls

HLA	Prostitutes			Total no. infected (n = 118)	Total no. resistant (group 4, n = 30)	Controls (n = 238)
	With gonorrhoea (group 1, n = 56)	With syphilis (group 2, n = 31)	With gonorrhoea and syphilis (group 3, n = 31)			
A1	0	6.5	0	1.7	0	0
2	62.5	58.1	61.3	61.0	30.0*	52.9
3	0	3.2	3.2	1.7	3.3	0.4
9	32.1	32.3	35.5	33.1	43.3	27.3
10	5.4	3.2	9.7	5.9	6.7	5.0
11	48.2	58.1	45.2	50.0	60.0	60.5
28	1.8	0	0	0.8	0	0.4
29	1.8	0	3.2	1.7	3.3	1.3
AW19	17.9	9.7	32.3	19.5	23.3	20.6
B5	10.7	9.7	9.7	10.2	13.3	12.6
7	1.8	0	3.2	1.7	3.3	1.7
8	0	0	0	0	0	0.4
12	1.8	3.2	0	1.7	6.7	3.4
13	16.1	16.1	16.1	16.2	23.3	20.2
14	0	0	0	0	0	0
15	32.1	29.0	22.6	28.8	33.3	22.3
17	17.9	12.9	25.8	18.6	13.3	14.3
18	0	0	0	0	0	1.7
27	3.6	6.5	5.1	5.1	0	7.1
37	1.8	0	0	0.8	0	0.4
40	36.4	35.5	32.3	34.7	38.7	40.0
BW16	16.1	16.1	16.1	16.1	9.7	10.0
21	0	0	0	0	0	0
22	5.4	9.7	16.1	9.3	10.0	12.2
35	10.7	6.5	0	6.8	0	4.6
46	28.6	25.8	38.7*	30.5	13.3	22.7

*In comparison of patient group with controls $P < 0.05$

Table 4 Joint occurrences of locus A and B antigens in various groups of prostitutes and in the controls

Antigen	Prostitutes								Controls (n = 238)	
	With gonorrhoea (n = 56)		With syphilis (n = 31)		With gonorrhoea and syphilis (n = 31)		Resistant (n = 30)		No.	%
	No.	%	No.	%	No.	%	No.	%		
AW19 B17	5	8.9	2	6.5	8	25.8*	2	6.7	16	6.7
A11 B15	10	17.9	4	12.9	1	3.2	9	30.0†	32	13.4

* Difference between patient group and controls, $\chi^2 = 12.97$; $P < 0.0005$; relative risk = 4.83

† Difference between patient group and controls, $\chi^2 = 5.64$; $P < 0.025$; cross product = 2.76

associations may be detected by chance. In fact, after correction for the number of combinations tested (P value $\times$ 180) none of the P values are significant. Because the correct method of statistical analysis for this type of data, however, is by no means established, further studies are clearly necessary, since our study suggests that there may be HLA associations both in the susceptibility and in the resistance to venereal disease in prostitutes. The association appeared to be with the joint occurrence of A-locus and B-locus antigens. The frequency with which AW19 B17 occurred was significantly higher in prostitutes with both gonorrhoea and syphilis (25.8%) than in normal controls (6.7%) or other groups of prostitutes.

The joint occurrence of A11 B15 was more frequent among prostitutes who were resistant to disease (30%) than in controls (13.4%). The association of A11 B15 within the resistant group was even stronger when only those uninfected prostitutes who had been in business for over two years were included in the group (46.7%). On the other hand, the frequency with which A11 B15 occurred was very low among prostitutes with double infections (3.2%). It thus appears that A11 B15 was associated with a resistance to syphilis and gonorrhoea. Direct evidence of exposure to syphilitic and gonococcal organisms in this group of prostitutes would be difficult to obtain. This group of prostitutes operates in the same area as the other groups, and all the prostitutes in this group had been in business for more than a year, (15 (50%) out of 30 for more than two years and 10 (33%) out of 30 for more than five years). Singapore is also a major port and tourism is one of its main industries. It seems likely, therefore, that this group had been exposed to syphilis and gonorrhoea.

Confirmation of the joint occurrences of A-locus and B-locus antigens would suggest that the genes

which influence disease susceptibility and resistance are present in linkage disequilibrium with the major histocompatibility locus genes. These genes may be immune-response genes modifying the host's response to these sexually transmissible organisms, though other possibilities, such as genes which regulate the biochemical factors in vaginal secretions which prevent bacterial colonisation—for example, pH or lysozyme content—must be considered. If IR genes are concerned it may be possible to confirm this hypothesis by using assays of cellular immune response to gonococcal and treponemal antigens *in vitro*. Though these results may not have immediate clinical significance, it is possible that they could lead to new immunotherapeutic approaches to infectious diseases in general.

This study was supported in part by research funds from WHO IRTC Singapore. The authors wish to thank Drs Betuel, Dausset, Hayes, Joysey, Thorsby, and Payne and the NIH serum bank for kind donations of HLA typing sera.

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Clinical pharmacological studies of amoxycillin: effect of probenecid

R. BARBHAIIYA, R. N. THIN, P. TURNER, AND J. WADSWORTH

From the Departments of Clinical Pharmacology and Genital Medicine and the Computing Unit for Medical Sciences, St Bartholomew's Hospital, London

SUMMARY In a study of eight, healthy, adult volunteers given 3 g amoxycillin with or without 1 g probenecid, significantly higher peak plasma levels of amoxycillin were recorded in the presence ($34.96 \mu\text{g/ml}$) of probenecid than in its absence ($22.72 \mu\text{g/ml}$). When plasma levels were plotted against time the mean area under the curve was significantly greater for subjects given probenecid than for those given amoxycillin alone. These findings suggest that 3 g amoxycillin plus 1 g probenecid provide better bioavailability than 3 g amoxycillin alone. The plasma levels obtained were several times higher than the minimum inhibitory concentrations (MICs) of most strains of gonococci. Plasma levels in excess of the MICs for most strains were maintained for eight hours with both regimens, but the higher levels in the presence of probenecid support the better clinical results previously reported with this regimen.

Introduction

Amoxycillin is a semisynthetic penicillin similar in chemical structure and spectrum of activity to ampicillin (Sutherland *et al.*, 1972) but is much better absorbed after oral administration (Croydon and Sutherland, 1971; Gordon *et al.*, 1972). Probenecid competes with penicillins and other organic anions for renal secretion, resulting in higher plasma levels of penicillin when they are administered together (Burnell and Kirby, 1951; Vitti *et al.*, 1974). Probenecid has been used together with ampicillin and amoxycillin for single-dose therapy for gonorrhoea (Karney *et al.*, 1974; Thin *et al.*, 1977). To investigate this further, plasma levels achieved after a dosage of 3 g amoxycillin with or without concurrent administration of 1 g probenecid were estimated in healthy volunteers. In view of the uncertain fasting state of patients receiving the antibiotic therapy for gonorrhoea and the small effect of food on plasma levels of amoxycillin (Neu and Winshell, 1971) the kinetic study was carried out in non-fasting healthy volunteers.

Materials and methods

The clinical pharmacological studies were conducted in eight healthy adult volunteers (age 22-26 years, weight 60-80 kg), all of whom gave informed consent. All subjects were shown by medical examination to be in good physical condition with normal blood and urine laboratory values. No subject had a history of allergic reactions to penicillins or cephalosporins. No other medication was taken by the subjects during the study.

Four subjects received 3 g ($1 \text{ g} \times 3$) amoxycillin with 1 g probenecid, and the other four received the same medication without probenecid. One week later the subjects were given the treatment they had not received during the first week. The treatment was administered orally with 100 ml water at least two hours after a standard breakfast of fruit juice and toast, and no food was allowed thereafter for four hours.

Blood samples were collected just before (zero hour) and at $\frac{1}{2}$, 1, $1\frac{1}{2}$, 2, 3, 5, 6, and 8 hours after the antibiotic administration. Plasma samples obtained were kept frozen at -20°C until assayed by a fluorimetric assay (Barbhaiya *et al.*, 1977). A plot of the time course versus plasma levels of amoxycillin with or without probenecid was obtained for each subject, and from this the area under the curve (AUC) between zero and eight hours was calculated.

Address for reprints: Professor P. Turner, Department of Clinical Pharmacology, St Bartholomew's Hospital, London EC1A 7BE

Received for publication 6 October 1978

The AUC was taken as a measure of relative absorption of amoxycillin in the absence and presence of probenecid. Paired *t* tests were used to assess the significance of within-subject differences and the optimal concentration/time curves were calculated for each subject using a least-squares, fitting technique.

Results

Plasma concentrations of amoxycillin achieved in each subject after administration of a single oral dose of 3 g amoxycillin with or without concurrent administration of 1 g probenecid are presented in Table 1. In the absence and presence of probenecid mean peak plasma amoxycillin levels were achieved at three hours and ranged from one to four hours. The mean peak plasma level for amoxycillin was significantly higher when probenecid was administered ($34.96 \mu\text{g/ml}$) than that achieved without administration of probenecid ($22.72 \mu\text{g/ml}$) ($t = 3.62$; $P < 0.01$). The peak plasma concentrations ranged from 23.20 – $62.00 \mu\text{g/ml}$ in the presence of probenecid and from 15.34 – $32.46 \mu\text{g/ml}$ in its absence (Table 1).

The mean area under the curve was significantly higher in the presence of probenecid than that obtained in its absence in each of the eight subjects (paired *t* test = 6.13 ; $P < 0.001$). The mean AUC without probenecid was 128.93 ± 6.13 and with probenecid 208.55 ± 9.22 . The AUC ranged between 170.84 – 233.29 and 115.65 – 165.66 in the presence and absence of probenecid respectively (Table 2).

Table 2 Area under the concentration/time curve for amoxycillin with and without probenecid

Subject no.	Area under concentration/time curve for amoxycillin	
	With probenecid	Without probenecid
1	233.29	115.65
2	170.84	165.66
3	242.24	130.63
4	222.14	139.94
5	225.90	109.69
6	179.69	114.84
7	199.74	139.99
8	199.64	115.29
Mean $\pm$ SEM	208.55 ± 9.22	128.93 ± 6.72

Discussion

Several studies have compared the clinical pharmacology of amoxycillin and ampicillin after oral administration and have uniformly demonstrated significantly better absorption levels of amoxycillin (Gordon *et al.*, 1972; Sutherland *et al.*, 1972). The plasma levels of these antibiotics are considerably raised in the presence of probenecid. The present study shows that 3 g amoxycillin achieves higher plasma levels in the absence of probenecid than those reported for 3.5 g ampicillin with 1 g probenecid and equivalent peak levels to those reported for 2 g amoxycillin with 0.5 g probenecid (Vitti *et al.*, 1974). The mean plasma levels obtained in eight subjects given 3 g amoxycillin

Table 1 Concentration of amoxycillin in plasma of non-fasting volunteers after oral administration

Subject no. and regimen	Antibiotic concentration ($\mu\text{g/ml}$) at given time intervals (hours)							
	$\frac{1}{2}$	1	$1\frac{1}{2}$	2	3	4	6	8
Amoxycillin 3 g								
1	6.47	9.96	15.19	13.45	19.72	18.68	8.56	4.50
2	3.15	8.20	18.5	29.32	32.46	27.60	11.00	5.45
3	12.47	23.11	30.52	28.42	26.36	19.40	6.00	4.30
4	1.46	6.34	15.80	16.90	28.86	23.16	12.72	4.60
5	1.47	5.05	11.23	12.60	15.34	13.25	10.80	6.97
6	3.70	10.80	12.90	16.74	18.84	20.23	9.42	3.00
7	3.80	9.40	16.80	19.50	24.76	21.20	12.30	5.30
8	2.90	8.30	14.19	16.15	20.40	18.67	9.00	4.60
Mean $\pm$ SEM	4.46 ± 1.2	10.45 ± 1.96	16.90 ± 2.10	19.13 ± 2.25	22.72 ± 1.57	20.27 ± 1.45	9.98 ± 0.77	4.46 ± 0.59
Amoxycillin 3 g and probenecid 1 g								
1	2.10	12.20	19.20	23.40	34.53	32.45	20.54	14.00
2	8.68	30.14	35.92	43.00	34.40	22.11	12.17	5.00
3	8.00	29.50	49.00	62.00	51.60	32.00	14.95	7.50
4	2.30	5.80	9.40	13.95	25.50	28.95	18.83	13.95
5	2.65	6.62	12.40	14.50	32.00	33.50	18.50	13.50
6	6.71	12.95	18.45	22.41	26.68	23.20	16.50	10.00
7	8.50	20.22	31.72	36.21	38.40	26.25	14.60	8.70
8	4.28	13.92	19.79	28.60	36.62	28.92	16.12	9.79
Mean $\pm$ SEM	5.4 ± 1.0	16.40 ± 3.32	24.49 ± 4.69	30.51 ± 5.72	34.96 ± 2.60	28.42 ± 1.50	16.50 ± 0.97	10.31 ± 1.17

and 1 g probenecid were considerably higher than those achieved without concurrent administration of probenecid. After administration of 500 mg and 1000 mg amoxycillin, peak levels of 7.9 µg/ml and 12.0 µg/ml respectively after one hour have been reported (Verbist, 1974; Spyker *et al.*, 1977). Vitti *et al.* (1974), however, reported 23.1 µg/ml and 21.4 µg/ml peak plasma levels at three hours after administration of 2 g amoxycillin with 0.5 g probenecid and 3 g amoxycillin alone respectively. In the same study the plasma concentration at eight hours with 2 g amoxycillin and 0.5 g probenecid combined was 3.8 µg/ml whereas that of 3 g amoxycillin was 1.8 µg/ml. Probenecid, therefore, appeared to be blocking renal excretion and prolonging plasma levels. The mean peak plasma levels observed during the present investigation (22.7 µg/ml at three hours with 3 g amoxycillin without probenecid) is in close agreement with the findings of Vitti *et al.* (1974). In the present study, however, a mean peak level of 4.46 µg/ml amoxycillin was observed at eight hours for the dosage regimen without probenecid compared with that of 1.80 µg/ml reported by the same author. Significantly higher peak plasma levels and area under the curves obtained for 3 g amoxycillin with 1 g probenecid than those for 3 g amoxycillin alone suggest better bioavailability with the former dosage regimen. The plasma levels were several times higher than the MIC values of most strains of gonococci sensitive to amoxycillin.

A correlation between low plasma levels, relative penicillin resistance, and increased failure rates in the treatment of gonorrhoea with previously adequate antibiotic regimens has been well documented (Eisenstein, 1977). Probenecid was introduced, therefore, to achieve a cure rate of over 95%. Eagle *et al.* (1948) emphasised the importance of the duration of effective penicillin bactericidal levels, based on the fact that the cells need to be growing in order to be affected. Eriksson (1971) reported that it took at least five, and as long as 12, hours of bactericidal concentrations of ampicillin to cure

gonorrhoea. Thus, the duration of antibiotic levels as well as their magnitude are important. The success of the recently conducted clinical trial for the treatment of gonorrhoea using a single oral dose of 3 g amoxycillin with 1 g probenecid (Thin *et al.*, 1977) may be attributed, at least in part, to the higher and persistent plasma levels observed with such a dosage regimen.

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Haematuria presenting in outpatients attending a department of genitourinary medicine

K. L. AMARASURIYA

From the Department of Genitourinary Medicine, Guy's Hospital, London

SUMMARY Of all the patients attending a department of genitourinary medicine during a 10-month period, about 2% (1 out of 50) presented with haematuria, or haematuria was discovered on initial examination. In about 25% of cases, the haematuria was due to *Escherichia coli* infection of the lower genitourinary tract. Gonococcal infection was the next commonest cause; one patient with gonorrhoea presented with frank urethral bleeding. In the remaining patients other causes of haematuria, which included renal cyst, carcinoma of the ureter, bilharziasis, and IgA disease, required more extensive investigations and follow up.

Introduction

The spectrum of disease in a department of genitourinary medicine covers infective and non-infective disorders. Since these departments were for the most part established for the clinical investigation of infectious disease, it is of interest to report the incidence of haematuria which occurred in patients attending over a 10-month period. None of these patients had been investigated elsewhere. This paper describes the type of presentation and the investigations undertaken in 12 such cases.

Methods

The basic investigations which were carried out in each case were: urethral smear (for Gram staining and microscopy); urethral culture (for detection of gonococci and anaerobic bacteria); mid-stream urine analysis (for detection of casts, leucocytes, red blood cells, protein, and glucose) and culture for micro-organisms; centrifuged deposit of urine (for Gram staining and culture). The following investigations were done in some cases, if indicated: intravenous urography (IVU), urethroscopy, serum biochemical analysis, and ASO titre. In special cases, urine was examined for tubercle bacilli or *Bilharzia*.

Results

The causes of haematuria in the 53 patients investigated are given in the table.

Address for reprints: K. L. Amarasuriya, Department of Genitourinary Medicine, Guy's Hospital, London SE1

Received for publication 21 August 1978

Table Causes of haematuria in 53 patients investigated

Cause	No. of cases
<i>Escherichia coli</i> urinary infection	25
Gonorrhoea	7
Abacterial urethritis	6
Renal calculi	4
Mycoplasmal urethritis	3
Tumour	2
Cryptogenic	2
Renal cyst	1
Ureterocoele	1
Tuberculosis	1
Bilharziasis	1

CASE HISTORIES

Case 1

A married Englishman, aged 23, presented with a history of haematuria and urethral discharge for one day. He cohabited with his wife. Clinical findings were: purulent and blood-stained urethral discharge; *Neisseria gonorrhoeae* detected in urethral smear and culture; urine analysis showed first and second glass haze and RBC +; and mid-stream urine showed WBC +, RBC +, and no growth on culture. The patient was treated with 2.4 megaunits procaine penicillin intramuscularly plus 2 g oral probenecid and made an uneventful recovery. Urethral gonorrhoea was diagnosed.

Case 2

A married Englishman, aged 46, presented with a history of haematuria and urethral discharge for three days. He had had marital intercourse five days previously and intercourse with an unknown girl three weeks previously. Clinical findings were: purulent urethral discharge and pus cells (+ +) in urethral smear; blood-stained urine showing first and second glass haze with RBC (+) and protein (+); and

centrifuged urine deposit containing pus cells (+) and coliform organisms on Gram stain; mid-stream urine showed WBC 1330 μl , RBC 900/ μl , and >100 000 *E.coli*/ml, resistant to ampicillin and amoxycillin but sensitive to doxycycline, gentamicin, and trimethoprim. The patient responded satisfactorily to treatment with triple tetracycline. *E.coli* infection of the lower genitourinary tract was diagnosed.

Case 3

A married Bangladeshi man, aged 55, presented with a history of haematuria and urethral discharge since the previous night, together with fever and rigors. He had had no sexual intercourse for five years. Coincidental findings were: temperature 38.5°C; purulent and bloody urethral discharge with coliform organisms on Gram stain; urine showed second glass haze and a positive result for blood on routine analysis; full blood count showed increased WBC with neutrophilia, ESR 19 mm in first hour; and mid-stream urine specimen contained WBC 460/ μl , RBC 400/ μl , and 100 000 *E.coli*/ml, sensitive to amoxycillin, ampicillin, gentamicin, trimethoprim, and doxycycline. Intravenous urography was normal. The patient was treated with amoxycillin, 500 mg four times daily for one week, and became asymptomatic. *E.coli* infection of the lower genitourinary tract, with constitutional symptoms, was diagnosed.

Case 4

A single Englishman, aged 17, presented with a history of haematuria and of urethral discharge for two weeks, for which he had been given Septrin by his own doctor. He had had sexual intercourse with an unknown girl four weeks previously. Clinical findings were: purulent urethral discharge; urine showed second glass haze with blood (RBC +) and protein (a trace); and mid-stream urine specimen contained WBC 3690/ μl , RBC 480/ μl but no growth on culture. Large-colony mycoplasmas were found on culture from urethral discharge. No abnormality was found in full blood count, ESR, ASO titre, or throat swab. Intravenous urography suggested a small cyst in the left kidney but was otherwise normal. Ultrasonic scan suggested a small cyst in the upper pole of the left kidney. The patient recovered after treatment with triple tetracycline and was referred to the genitourinary surgeon. Renal cyst and mycoplasma-positive urethral discharge was diagnosed.

Case 5

A single Rhodesian man, aged 20, presented with haematuria and urethral discharge. He gave a history of bilharziasis (vesical) treated three years earlier in Rhodesia, where he had lived until three months before presenting here. Clinical findings were: purulent urethral discharge containing coliform organisms on Gram stain; blood-stained urine showed first glass haze and second glass haze; centrifuged urine deposit contained WBC, RBC, and coliforms; and mid-stream urine showed WBC 14/ μl , RBC 200/ μl , but no growth of micro-organisms. Microscopical examination of terminal strained urine gave negative results for *Bilharzia*, ova, and cysts. The following investigations all gave negative results or were within normal limits: full

blood count and ESR, blood urea and serum electrolytes, ASO titre, throat swab, Mantoux 1/10 000, x-ray examination of chest, and intravenous urography. The patient was treated with triple tetracycline, and a week later the discharge had cleared and prostatic examination was normal; the haematuria (on microscopical examination), however, persisted. Further laboratory examination of strained terminal urine demonstrated the presence of *Schistosoma* ova. Cystoscopy was advised, but the patient left the country without keeping the appointment and did not return. Bilharziasis with haematuria was diagnosed.

Case 6

A single Englishman, aged 29, presented with a history of urethral discharge and haematuria for three days, and passing renal stones intermittently for the past three years. He had sexual contact only with his wife. There was a previous history of coeliac disease in childhood, treated with an appropriate diet, and of pulmonary tuberculosis in 1958, treated in Ireland. His mother had had tuberculosis, his father diabetes and renal stones, and his sister renal stones. Urine examination showed haematuria on microscopical examination, no acid-fast bacilli on Ziehl Neelson stain, and no bacteria on culture after eight weeks incubation on several occasions. Mid-stream urine specimens contained no WBC; RBC was 200/ μl ; no growth of organisms occurred on culture. Blood urea and serum electrolytes, urine oxalate, and blood glucose were all within normal limits. Intravenous urography showed renal calculi in the left kidney, and the patient was referred to a surgeon. There was no sign of tuberculosis. Renal calculi were diagnosed.

Case 7

A single West Indian man, aged 21, presented with a history of haematuria and urethral discharge for five days. Clinical findings were: purulent urethral discharge; blood-stained urine showing first glass haze with shreds and second glass haze with RBC; and the centrifuged deposit contained WBC. *Ureaplasma urealyticum* and *Mycoplasma hominis* but no chlamydiae were isolated from the urethral discharge. Mid-stream urine showed no growth of organisms. This patient's condition cleared normally on treatment with triple tetracycline. Endoscopy was reported as normal. Mycoplasma-positive urethritis was diagnosed.

Case 8

A married Englishman, aged 26, presented with a history of lower backache for two months and dark urine for four days. He cohabited with his wife. On examination, there was no urethral discharge. Urine showed first glass haze and second glass haze with RBC; centrifuged deposit contained no WBC and no organisms. The following investigations were all normal: mid-stream urine, full blood count and ESR, ASO titre, and throat swab. The IVU showed a large 'spring onion' defect in the bladder on the right side, indicating a large ureterocoele which was causing obstruction to the entire collecting system on that side and resulting in haematuria. The left kidney was normal, but there was some loss of cortex on the right side. The patient was referred to a genitourinary surgeon. A ureterocoele was diagnosed.

Case 9

A married Englishman, aged 66, presented with a history of periodic epigastric pain and a dull, almost continuous, mainly right-sided abdominal pain radiating to the back for the past two years. On examination, there was no urethral discharge. Blood pressure reading was 140/80 mmHg. Urine showed first glass clear and second glass clear with microscopic haematuria. MSU contained no WBC, RBC 130/ μ l, and no growth of organisms. Full blood count and ESR, blood urea, serum electrolytes, serum amylase, ASO titre, and throat swab were all normal. Barium meal examination showed a healed duodenal ulcer. The IVU showed a lesion of the right ureter; this proved to be a carcinoma which was removed surgically and the ureter transplanted into the bladder. The haematuria cleared and the patient made an uneventful recovery. Carcinoma of the ureter was diagnosed.

Case 10

A single English boy, aged 15, was referred from the urology department with a history of persistent haematuria over the past three years and occasional frank bleeding. There had been intermittent dysuria for three months. There was no history of sexual intercourse and no history of masturbation. On examination, there was a clear urethral discharge and a small abrasion on the inner, left aspect of the meatus. The urethral smear contained pus cells. No mycoplasmas or chlamydiae were isolated. Darkground microscopy showed negative results for treponemes and *Trichomonas vaginalis*. Herpes virus was cultured from the meatal lesion. The first glass urine was clear with shreds and the second glass was clear with RBC. Mid-stream urine contained WBC 14/ μ l, RBC 20/ μ l, but no growth on culture. No abnormality was found on IVU, micturating cystogram, urethroscopy, cystoscopy, or prostatic examination. Full blood count and ESR, blood urea and serum electrolytes, ASO titre, and throat swab were all within normal limits. The non-specific urethritis responded to treatment with triple tetracycline, but the microscopic haematuria persisted over the following two months and was considered to be due to capillary bleeding in the posterior or membranous urethra. Non-specific urethritis (not sexually mediated) and cryptogenic haematuria were diagnosed.

Case 11

A single Englishman, aged 20, presented with a history of haematuria for three days together with bilateral aches in the loins. On examination there were no lower genitourinary tract signs apart from macroscopic and microscopic haematuria. His blood pressure was normal, and there was no history of previous streptococcal infection or of present or previous joint involvement. He had been seen in this clinic six weeks previously with non-specific urethritis. He also gave a history of haematuria nine months previously, which was investigated by his general practitioner and confirmed by mid-stream urine. An IVU performed at that time was normal. Investigations on this occasion showed: IVU normal; mid-stream urine specimen containing RBC but no growth of organisms. The following tests all gave negative results or were within normal limits: ASO titre, throat swab, autoantibodies, urine cultures for

tubercle bacilli successively, blood urea and electrolytes, prothrombin and clotting time, full blood count and ESR, Paul Bunnell, x-ray examination of chest, and ECG. Renal biopsy showed a mild mesangial proliferative glomerulonephritis with IgA deposits. It is of interest that a similar patient, in whom IgA disease was also diagnosed, presented during the previous year in this department. IgA disease was diagnosed.

Case 12

A single African man, aged 24, presented with a urethral discharge and dysuria. He had had sexual intercourse only with a regular consort. Clinical findings were: purulent urethral smear; first and second glass urine contained haze, shreds, and RBC; and centrifuged deposit contained pus cells. Mid-stream urine analysis showed no growth of organisms. No bacterial pathogens, no chlamydiae, and no mycoplasmas were isolated on urethral culture. The condition cleared after a five-day course of triple tetracycline. Abacterial haematuria was diagnosed.

Discussion

The manifold causes of haematuria are well known (Hart, 1973; Lytton, 1977; Higgins, 1978). Lee and Davis (1953) found that most of the 1000 cases of haematuria investigated by them could be classified under six common causes (in descending order of frequency): cystitis, tumour of the bladder, benign hypertrophy of the prostate, chronic prostatitis, essential (of doubtful aetiology) haematuria, and calculus of the ureter. According to Boyd (1977) three common causes—namely, renal and urothelial malignancy, infection, and calculus disease—together accounted for most diagnoses while most of the remaining cases of haematuria were due to glomerulonephritis, renal cystic disease, trauma, infarction, and embolism. Lytton (1977) states that in his experience 'the commonest cause of haematuria is acute cystitis'.

It is of interest that out of the many female patients attending this department with *E.coli* infection of the lower genitourinary tract during the period of the present study none presented with haematuria whereas over three-quarters of the male patients with *E.coli* infection did have haematuria. Where blood was found in the urine of female patients, in almost every case it was due to contamination (for example, menstruation). In one female patient, however, who presented with vaginal discharge and haematuria the latter was due to urethral gonorrhoea.

The case of bilharziasis reported above is a reminder that, especially in these days of increased travel, such conditions need to be considered as possible causes of haematuria.

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Seasonal variations in the reported incidence of sexually transmitted diseases in Scotland (1972-76)

C. B. S. SCHOFIELD

From Newcastle General Hospital, Newcastle upon Tyne

SUMMARY The seasonal variation in the quarterly incidence of some sexually transmitted diseases and other conditions in Scotland is compared with that of gonorrhoea and of conceptions leading to live births or abortions. The seasonal incidence for non-specific genital infections and for other conditions not requiring treatment in both men and women was similar to that of gonorrhoea, thus indicating an association with promiscuity, whereas the seasonal variation for candidosis in men and women—an infection not usually associated with promiscuity—was similar to that of conceptions. Differences between the sexes occurred, however, in the seasonal incidences of scabies, pubic lice, genital herpes, trichomoniasis, and other conditions requiring treatment; thus men with these conditions appeared to be more promiscuous than women. The seasonal variations in incidence of genital scabies and pubic lice indicate that these infestations are more easily transmitted by close bodily contact indoors during cold weather than in the open air.

Introduction

Most venereologists in Britain are well aware that the busiest month in their clinics is August. Cornelius (1971) reported that the quarterly incidence of gonorrhoea in the United States was 94, 97, 110, and 100% (100% = that expected if attendance was the same in each quarter). Similar seasonal variations have been noted in the reported incidence of gonorrhoea in Scotland but not in that of all the other sexually transmitted diseases nor in the quarterly incidence of abortions or live births.

About 65% of gonococcal infections in Scotland are acquired as a result of indiscriminate, promiscuous, sexual intercourse (Schofield, 1975). This finding has formed the basis for a hypothesis that the seasonal variations in the reported incidence of the venereal diseases (those mainly acquired as a result of indiscriminate promiscuity) would be similar to those of gonorrhoea—given a fairly constant incubation period—whereas the non-venereal diseases (those not usually associated with promiscuity) would have seasonal variations in incidence similar to those of conceptions leading to live births or abortions.

Method

STATISTICS

Information on the number of patients attending special clinics was obtained from the quarterly statistical returns (ISD(D)5) submitted to the Scottish Home and Health Department for the years 1972-76 inclusive. Live birth statistics were obtained from the annual report of the Registrar General for Scotland (1976) and the abortion statistics from the Scottish Home and Health Department's (1973-76) published figures, in both cases for the years 1971-76 inclusive. To estimate the time of conceptions leading to abortions the published figures were adjusted back 2½ months, the average duration of pregnancies before therapeutic abortions being between 10 and 11 weeks, while those for live births were adjusted back nine months. The quarterly incidences are recorded as percentages of those expected, had an equal number of patients attended each quarter (Figure) and the annual pattern is repeated to show the continuity between the fourth and first quarters.

Results

GONORRHOEA

The seasonal incidence of genital gonorrhoea in men and women was similar, with the maximum attendance in the third quarter and the minimum in

Address for reprints: Dr C. B. S. Schofield, Ward 34, Newcastle General Hospital, Westgate Road, Newcastle upon Tyne NE4 6BE

Received for publication 10 July 1978

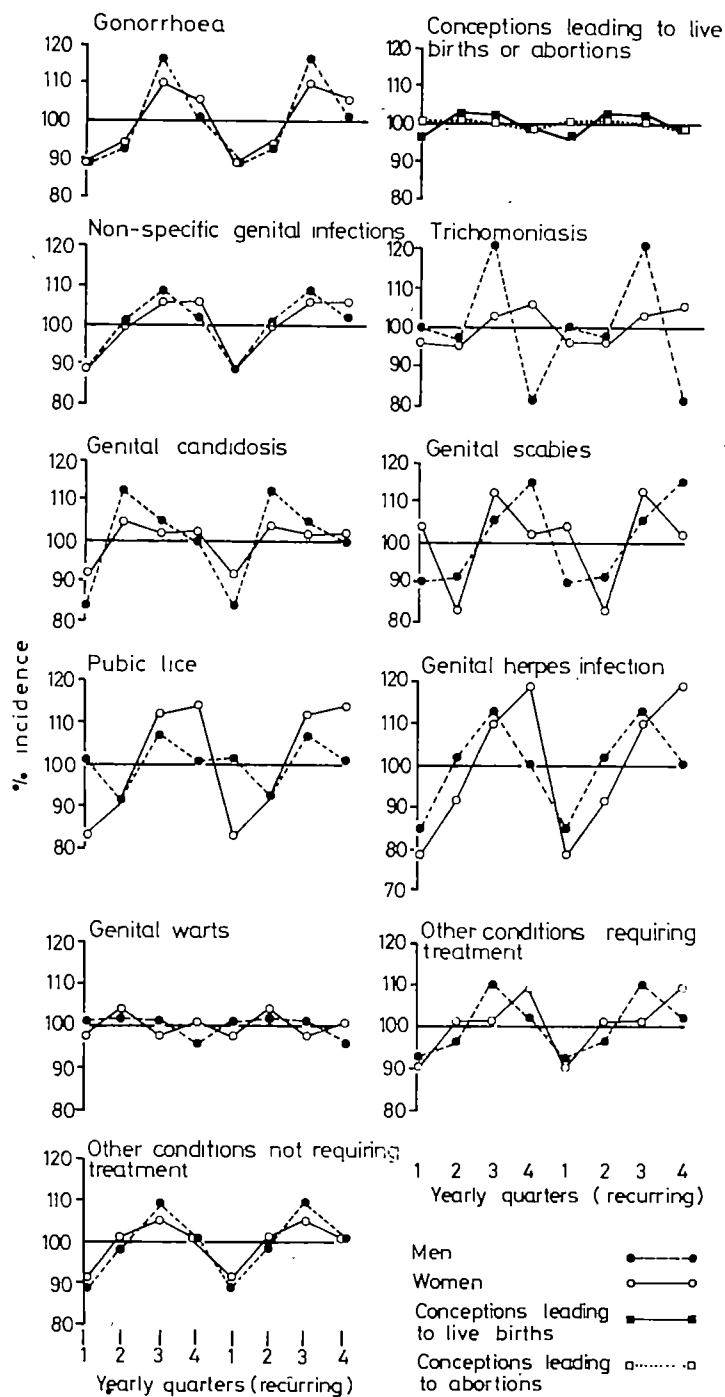


Figure Percentage quarterly incidence of gonorrhoea, conceptions leading to live births or abortions, non-specific genital infections, trichomoniasis, candidosis, scabies, pubic lice, herpes, warts, other conditions requiring treatment, and other conditions not requiring treatment. (The annual pattern is repeated to show the continuity between the fourth and first quarters.)

the first, the ranges being 27.8% and 20.8% respectively (Figure and Tables 1 and 2).

CONCEPTIONS

The seasonal incidence of conceptions leading to live births was similar to that of conceptions leading to therapeutic abortions (Figure and Tables 1 and 2). The maximum incidence of both occurred in the second quarter decreasing to a minimum in the fourth, while the ranges were only 5.5% and 2.8% respectively, thus giving different patterns from those of gonorrhoea.

NON-SPECIFIC GENITAL INFECTIONS

The seasonal incidence of non-specific genital infections (Figure) was similar to that of gonorrhoea, with the maximum attendance in the third quarter and the minimum in the first, the ranges being 19.7% and 16.8% respectively (Table 1).

TRICHOMONIASIS

The number of men with trichomoniasis was very small (Table 1), but the seasonal incidence was an exaggeration of that of gonorrhoea, with a range of 39.9% (Figure). The seasonal incidence of trichomoniasis in women differed from that of gonorrhoea and of conceptions, with the maximum attendance in the fourth quarter and the minimum in the second, the range being 10.3%.

CANDIDOSIS

The seasonal incidence of genital candidosis in men and women was similar and quite unlike that of gonorrhoea (Figure). With maximum attendance in the second quarter and minimum in the first (ranges of 29.6% and 13.2% respectively) the patterns were exaggerations of those of conceptions (Table 1).

SCABIES

Only 169 cases of genital scabies in women were recorded over the five-year period (Figure and Table 1). Maximum attendance was in the third quarter but another peak occurred in the first, the range being 28.5%. The seasonal incidence in men—with minimum attendance in the first two quarters and maximum in the fourth—did not resemble that of gonorrhoea or of conceptions, the range being 24.6% (Table 1).

PUBIC LICE

Few cases of pubic lice occurred in women (Figure and Table 1); the minimum attendance occurred during the first quarter and the maximum in the third and fourth, with a range of 30.9%. The seasonal incidence of pubic lice in men was similar to that of gonorrhoea, with minimum attendance in the second quarter, the range being 14.5%.

Table 1 *Number of attendances by men and women with sexually transmitted diseases*

Diagnosis	Attendances		% range between maximum and minimum attendances	
	Men	Women	Men	Women
Gonorrhoea	15 558	9251	27.8	20.8
Conceptions leading to live births		327 005		5.5
abortions		36 353		2.8
Non-specific genital infections	22 687	2201	19.7	16.8
Trichomoniasis	261	7899	39.9	10.3
Candidosis	1512	6337	29.6	13.2
Scabies	960	169	24.6	28.5
Pubic lice	1840	320	14.5	30.9
Genital herpes	1436	360	27.6	40.0
Genital warts	6646	2755	6.4	7.0
Other conditions requiring treatment	16 420	4157	19.2	18.3
Other conditions not requiring treatment	17 323	8014	20.3	14.1

Table 2 *Range of seasonal incidence for gonorrhoea and conceptions between men and women*

'Control' conditions	% range of incidence (by yearly quarters)			
	First	Second	Third	Fourth
Gonorrhoea	88.9-89.3	93.1-95.0	110.1-116.7	101.3-105.6
Conceptions	97.0-100.0	101.2-102.5	100.4-101.7	98.4-98.8

GENITAL HERPES

Again, few cases of genital herpes infections occurred in women (Figure and Table 1), and the seasonal incidence was unlike that of either gonorrhoea or conceptions, with the minimum attendance in the first and the maximum in the fourth quarters, a range of 40.0%. The seasonal incidence of genital herpes infection in men, however, was similar to that of gonorrhoea, with a range of 27.6%.

GENITAL WARTS

Because of the wide variation in the incubation period of genital warts there was no valid pattern in the seasonal incidence for either sex (Figure and Table 1). The number of male attendances during the first three quarters was steady, with the minimum during the fourth, while the number of female attendances fluctuated from one quarter to the next (Table 1); the ranges were 6.4% and 7.0% respectively.

OTHER CONDITIONS REQUIRING TREATMENT

A wide variety of usually non-infectious and dissimilar clinical conditions of the genital areas are included under the heading of 'other conditions requiring treatment' (Table 1). Nevertheless, the seasonal incidence in men closely resembled that of gonorrhoea, with the maximum attendance during the third quarter and the minimum in the first, a range of 19.2% (Table 1). The seasonal incidence in women was different, however, with the minimum attendance in the first quarter and the maximum in the fourth, a range of 18.3% (Table 1).

OTHER CONDITIONS NOT REQUIRING TREATMENT

Most of the patients who attended and were found to be free of any sexually transmitted disease and in no need of treatment for any other condition (Table 1) came of their own accord having run a risk of infection. Far fewer attended either as sexual contacts of patients with a sexually transmitted disease and found not to be infected or because they believed, or feared, that they had a disease without being able to cite any particular risk. The seasonal incidence was very similar to that of gonorrhoea, with the maximum attendance during the third quarter and the minimum in the first, a range of 20.3% (Figure). The seasonal incidence for women was also similar, but the range was only 14.1%.

Discussion

A possible explanation for the incidence of diseases associated with promiscuity being at its highest in the

third quarter—the months of August, September, and October—has been proffered by Felton (personal communication). First pairings occur in the spring and second and casual ones in the summer; the original partners later rejoin, and about this time symptoms and signs become manifest in one or the other. The highest incidence of conceptions leading to live births or abortions in the second quarter might indicate that they occur mainly in first pairings, especially as there is no peak later in the year.

A similar pattern of disease-incidence for men and women was found in only three conditions, in addition to that of gonorrhoea: non-specific genital infections, other conditions not requiring treatment, and genital candidosis. The seasonal variation in the incidence of the first two conditions was similar to that of gonorrhoea, and this was to be expected. Most cases of non-specific genital infection were associated with promiscuous, sexual relationships by one or other partner, while most patients who attended for follow-up examinations did so after running a risk of infection with a casual partner. The seasonal variation in the incidence of genital candidosis was similar to, but an exaggeration of, that of conceptions. Willmott (1975) did not find any seasonal variation in the incidence of genital yeast infections in women, but he described only 207 cases. The seasonal variation in incidence of both genital candidosis and conceptions could reflect the varying frequency of sexual contact among the population at large, the former being an exaggeration and the latter an underestimation of the incidence; this would agree with the opinions of many clinicians that most cases of genital candidosis are not associated with sexual promiscuity.

For all other conditions differences in the seasonal incidence between the sexes were noted. In the case of genital scabies the minimum number of male attendances occurred during the first and second quarters, with a sharp increase in the third to a maximum number in the fourth. Very few women attended with genital scabies; maximum attendances occurred in the third quarter (which possibly reflects the rising number of infections in men) with a second peak in the first quarter—perhaps an aftermath of the peak in men in the fourth quarter. The high incidence of infections occurring during the cold months might indicate that this infestation is more readily transmitted by close bodily contact indoors than in the open air.

The seasonal incidence of all other conditions in men was similar to that of gonorrhoea, indicating an association with a high rate of sexual promiscuity. The highest incidences of genital herpes and other conditions requiring treatment occurred during the third quarter, with the lowest incidence in the first,

whereas the highest incidences of trichomoniasis and pubic lice in men occurred in the fourth and second quarters respectively.

The seasonal incidence of these conditions in women was usually lowest during the first quarter—that of trichomoniasis being lowest during the second—with the incidence rising sharply by the third to a peak by the fourth quarter. Neilson (1973) has reported an increase in the frequency with which *Trichomonas vaginalis* was cultured during the autumn (September, October, and November) in Copenhagen. The dissimilarity between the patterns of attendance of men and women with other conditions requiring treatment can be accounted for by the fact that very few were sexual partners, and they almost certainly represented two completely different populations. The same, however, cannot be said of those attending with infections, trichomoniasis, genital herpes, and pubic lice. As with scabies, over half the attendances by men and women with pubic lice occurred during the first and fourth quarters, which possibly indicates that these infestations are transmitted more easily by close,

bodily contact indoors rather than in the open air. Nevertheless, the most probable explanation for the maximum number of female attendances occurring one quarter after that of men is that little contact-tracing of patients with these conditions was carried out, most women only attending when symptoms had developed. While men with these conditions were often promiscuous, this was not necessarily true of women.

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Reinfection with disseminated gonococcal disease

NICHOLAS J. FIUMARA* AND A. DAVID SIMKIN†

*From the *Division of Communicable and Venereal Diseases, Massachusetts Department of Public Health, and †Boston City Hospital and Boston Dispensary, Tufts New England Medical Center, Boston, Massachusetts, USA*

SUMMARY A 20-year-old prostitute was admitted to hospital with joint pains and a macular and pustular rash on the fingers and feet on two occasions. Disseminated gonococcal infection was diagnosed and successfully treated.

Introduction

Disseminated gonococcal infection (DGI) occurs in 1% to 3% of patients whose exposure to infection occurred within the previous two months (Brooks *et al.*, 1976). The disease principally affects women. Reinfection with disseminated gonococcal disease is uncommon, and its incidence at this time is unknown. This paper reports such a case, which might add to the understanding of this disease.

Case report

On 26 May, 1977, a 20-year-old, Caucasian woman was admitted to a hospital in New York City complaining of pain in the wrists and a rash on the fingers and feet. She worked as a prostitute and was well until one week before admission. At that time she had a sore throat, low abdominal pain, and a vaginal discharge. She admitted to oral and genital contact but not anal. The patient attended a clinic where cervical specimens were taken for smear examination and culture; a blood specimen was taken for detection of syphilis antibodies. She was given nine pills to swallow and asked to return in a week's time. At this time she was told that the results of the investigations were all negative; although her throat was no longer sore the pain in the joints, fingers, and wrists was worse, and the evening before she had had attacks of shivering and a high fever. The patient was referred to the nearest hospital. The admitting physician recorded the history as above. On examination her temperature was 101°F; respiration

rate, 16/minute; pulse rate, 80/minute; and blood pressure reading, 100/65 mmHg. On the extensors of the fingers, hands, anterior surface of the lower legs, and dorsum of the feet there were discrete, macular and pustular lesions and a tender swelling of the right anterior wrist. There was a right lower abdominal scar which the patient stated was the result of a laparotomy in February 1976 because she had 'gonorrhoea of the tubes'. (This was subsequently confirmed.) Examination of the ear, nose, and throat showed only pharyngeal infection. The cervix was non-tender and freely moveable, with a mucoid discharge. The physician then took specimens for culture of the blood, pharynx, cervix, urethra, and anus. The blood and cervical cultures gave positive results for gonococci. The patient was treated with 12 megaunits crystalline penicillin G intravenously for four days. On the fifth day she was discharged and was given oral ampicillin, 2 g to be taken daily for the next two weeks. On discharge from hospital she no longer had a rash or joint pains.

The patient went back to work as a prostitute but came to Boston in February 1978 because she was pregnant; she planned to have her baby delivered in Boston. On the morning of admission to the clinic (February 1978), however, the patient again complained of pain in the wrists (in the same place as previously described), and a rash on the backs of her fingers, dorsum of the hand, upper forearms, and dorsum of the toes and lower legs. She recognised it as being similar to the rash she had had in the previous May and came to the clinic at the Boston Dispensary immediately. The rash was discrete and macular but there were also some small, centrally located vesicles, none of which were pustular or purpuric. The patient had had no attacks of shivering; her temperature was 98°F, pulse rate 76/minute, respiration rate 16/minute, and blood pressure reading 110/65 mmHg. She volunteered her

Address for reprints: Dr N. J. Fiumara, Division of Communicable and Venereal Diseases, Department of Public Health, 600 Washington Street, Boston 02111, Massachusetts, USA

Received for publication 5 July 1978

occupation and was accompanied by her consort. Specimens were taken for culture of the blood, pharynx, and cervix. Only the cervical culture gave positive results for gonococci; the rapid plasma reagin circle card (RPRCT) gave negative results. Her consort complained of no symptoms, but a calcium alginate swab of the urethra gave positive results on microscopical examination and later by culture. The contact was treated with 4·8 megaunits procaine penicillin G in aqueous suspension given intramuscularly. The patient was given the same dosage of penicillin to be followed the next morning with 2 g ampicillin to be taken daily for the next nine days. She returned on the fourth post-treatment day with no symptoms and no rash, but she was asked to complete the ampicillin treatment. Two weeks after finishing treatment the patient was clinically well and the cervical cultures gave negative results. This patient, therefore, had three documented gonococcal infections, two of them with bacteraemia.

Discussion

Acute disseminated gonococcal infection occurs in 1-3% of untreated patients. The onset is sudden, with shivering, fever, arthralgia, and tenosynovitis (particularly of the wrists and fingers). A rash subsequently appears on the peripheral areas (hands, fingers, feet, and toes) which at first consists of inflammatory macules, in the centre of which vesicles

form; these rapidly become pustular and then purpuric. Treatment is effective and the patient becomes free of symptoms within two or three days.

Patients with disseminated gonococcal infection may have a deficiency in complement, particularly C6, C7, or C8 (Handsfield, 1975; Petersen *et al.*, 1976). In view of this our patient was asked to return and a complement study was performed (by courtesy of Dr William McCormack, Channing Laboratory, Boston), which was normal.

A great deal needs to be known about immunity in gonorrhoea and host defence systems. With time and study these mechanisms will eventually be determined and then we can begin to understand the varied clinical syndromes of gonorrhoea (both asymptomatic and symptomatic), pelvic inflammatory disease, disseminated infection, and why some patients can be reinfected.

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Sexually transmitted diseases

Extract from the Annual Report of the Chief Medical Officer of the Department of Health and Social Security for the year 1977

The rate of increase in the incidence of most of the sexually transmitted diseases has slowed considerably since 1971 in England and in most other countries where figures are available. The two exceptions to this are syphilis and non-specific genital infection. The prevalence of the latter becomes greater each year despite a better understanding of some aspects of the condition. Recent research, moreover, has shown that complications of these diseases result in a considerable amount of chronic ill-health, and the economic consequences of this are only beginning to be studied.

Syphilis, gonorrhoea, and chancroid

Syphilis

Table 1 shows an increase in the incidence of early syphilis of 9.9% (9% among men and 15.2% among women). The incidence of primary and secondary syphilis increased by 4.9% (2.8% among men and 19.7% among women). The incidence rate for syphilis and gonorrhoea per 100 000 population is given in Table 2.

Gonorrhoea

All forms of gonorrhoea have shown a 1% increase in incidence (0.1% among men and 2.5% among women).

Chancroid

Cases of chancroid have decreased in number.

Other sexually transmitted diseases

The incidence of granuloma inguinale has increased in men (Tables 3 and 4). The number of cases of non-specific genital infection has increased in men and women, and so has that of genital herpes and genital warts.

The present position

The number of hospital medical staff engaged in genitourinary medicine in England and Wales on 30 September 1977 totalled 211 (188.6 whole-time equivalents (wte)) compared with 210 (185.1 wte) at

Table 1 Cases of syphilis, gonorrhoea, and chancroid reported in the year ending 30 June 1977, with figures for the year ending 30 June 1976 in parentheses (for incidence rates per 100 000 population see Table 2)

	Number of cases					
	Total		Men		Women	
Syphilis						
Early	2593	(2359)	2183	(2003)	410	(356)
Primary and secondary only	1735	(1654)	1486	(1446)	249	(208)
Late	1234	(1294)	821	(874)	413	(420)
Congenital	141	(141)	59	(63)	82	(78)
Gonorrhoea						
All forms	59 028	(58 444)	37 112	(37 069)	21 916	(21 375)
Post-pubertal						
All ages	58 987	(58 389)	37 100	(37 057)	21 887	(21 332)
Under 16 years	594	(577)	117	(108)	477	(469)
16-19 years	11 784	(11 467)	4745	(4628)	7039	(6839)
20-24 years	19 820	(19 321)	12 153	(11 895)	7667	(7426)
25-34 years*	19 112		13 823		5289	
35-44 years*	5852		4714		1138	
45 years and over*	1825		1548		277	
Chancroid	43	(68)	39	(62)	4	(6)

*Only one group (25 years and over) was collected before 1976.

Table 2 Syphilis and gonorrhoea—new cases per 100 000 population, by age, seen at hospital clinics in England 1973-77.

Disease	Years ending 30 June											
	1973			1974			1975			1976		
	Men	Women	Total	Men	Women	Total	Men	Women	Total	Men	Women	Total
Early syphilis												
All ages	6.84	1.31	4.00	8.57	1.46	4.92	8.44	1.48	4.87	8.86	1.50	5.08
Early syphilis (primary and secondary only)												
All ages	5.00	0.81	2.85	6.30	1.01	3.59	6.12	0.93	3.46	6.40	0.87	3.56
Under 16 years	0.07*	0.08*	0.07*	0.05*	0.05*	0.05*	0.05*	0.02*	0.04*	0.07*	0.09*	0.08*
16-19 years	5.43	2.90	4.20	7.01	3.55	5.32	6.54	3.76	5.18	7.52	3.30	5.46
20-24 years	14.67	4.76	9.77	18.16	4.26	11.29	18.02	4.60	11.42	19.35	3.46	11.58
25 years and over	5.94	0.50	3.06	7.52	0.81	3.98	7.26	0.65	3.77	7.40	0.68	3.85
Late syphilis												
All ages	3.41	1.51	2.43	3.51	1.51	2.48	3.68	1.73	2.68	3.87	1.76	2.79
Congenital syphilis												
All ages	0.28	0.39	0.34	0.21	0.42	0.32	0.32	0.46	0.39	0.28	0.33	0.30
Gonorrhoea (post-pubertal)												
All ages	157.30	80.94	118.08	169.60	88.92	128.19	163.67	87.62	124.66	163.91	89.60	125.79
Under 16 years	2.13	7.31	4.65	2.30	8.33	5.23	1.89	8.26	4.99	1.87	8.57	5.13
16-19 years	328.02	490.42	407.00	361.92	535.55	446.72	340.26	513.59	424.85	331.52	513.05	420.19
20-24 years	707.10	428.32	569.08	769.43	469.03	620.96	753.38	472.99	615.66	728.41	475.72	604.92
25 years and over	142.45	38.30	87.38	152.00	42.12	93.95	146.11	41.03	90.42	147.97	42.73	92.40
Chancroid												
All ages	0.20	0.00*	0.10	0.16	0.02*	0.09	0.18	0.03*	0.10	0.27	0.03*	0.15

*These rates were based on fewer than 10 events and consequently their reliability as a measure may be affected.

Table 3 *Other sexually transmitted diseases reported in the year ending 30 June 1977, with figures for the year ending 30 June 1976 in parentheses (for incidence per 100 000 population see Table 4)*

	Number of cases					
	Total		Men		Women	
Lymphogranuloma venereum	36	(33)	27	(31)	9	(2)
Granuloma inguinale	70	(16)	59	(12)	11	(4)
Non-specific genital infection (NSGI)	94 152	(87 686)	73 996	(70 163)	20 156	(17 523)
NSGI with arthritis	593	(491)	551	(468)	42	(23)
Trichomoniasis	19 988	(19 689)	1703	(1540)	18 285	(18 149)
Candidosis	36 731	(36 216)	6756	(6257)	29 975	(29 959)
Scabies	2238	(2650)	1768	(2128)	470	(522)
Pediculosis pubis	5838	(5201)	4081	(3634)	1757	(1567)
Genital herpes	7327	(6660)	4692	(4319)	2635	(2341)
Genital warts	22 385	(20 946)	14 648	(13 636)	7737	(7310)
Genital molluscum	915	(828)	614	(572)	301	(256)
Other treponemal diseases	1173	(1046)	746	(664)	427	(382)
Other conditions requiring treatment in a centre	39 960	(38 666)	25 833	(25 394)	14 127	(13 272)
Other conditions not requiring treatment in a centre	91 555	(86 068)	56 880	(53 690)	34 675	(32 378)
Other conditions referred elsewhere	1877	(858)*	1137	(542)*	740	(316)*

*These additional figures are for the quarters March and June 1976. Before 1976 these figures were not collected.

the previous 30 September. The 1977 figures included 106 (99.0 wte) consultants, 33 (32.6 wte) senior registrars, and 32 (30.2 wte) registrars compared with 103 (94.7 wte) consultants, 32 (30.0 wte) senior registrars, and 35 (31.5 wte) registrars on 30 September 1976. On 30 September 1977 there were also three (0.8 wte) hospital practitioners and 152 (35.6 wte) staff holding sessional appointments under paragraph 94 of the Terms and Conditions of Service. In Great Britain as a whole there were 115 consultants specialising in genitourinary medicine, of whom 85 were working whole-time.

After more than two decades of increasing incidence of most of the sexually transmitted diseases, some measure of control seems to have been established over them in England. The greater use of the Health Service and the higher expectations of the public, however, have resulted in an ever-increasing number of patients using the diagnostic and counselling services of the clinics. There has been a slight increase in trained staff to meet this demand, and recruitment to the expanding specialty of genitourinary medicine has improved slightly. It is most important for the specialty that the standard of training should be high.

London is now the leading centre in the world for postgraduate education in the sexually transmitted diseases. The twice-yearly postgraduate course organised by the British Postgraduate Medical Federation attracts doctors from all over the world and is always very well attended. A postregistration course, organised by the Joint Board of Clinical Nursing Studies, in the nursing of patients with sexually transmitted diseases lasts for six months and

is held at two London and two provincial teaching hospitals. In addition, many postgraduate students arrange attachments to individual teaching hospital clinics, through the World Health Organisation, the Department of Health and Social Security, the universities, or on an individual basis. The Diploma of Venereology awarded to successful candidates by the Society of Apothecaries is highly regarded throughout the world.

During 1977, the Medical Society for the Study of Venereal Diseases held its overseas spring meeting in Vienna, which was well attended by both European and British doctors. A session at the International Congress of Dermatology in Mexico City was devoted to syphilis, and a Canadian symposium on sexually transmitted diseases was held in Montreal. In addition, the First South-East Asian and Western Pacific Conference on Sexually Transmitted Diseases was held in Singapore under the auspices of the International Union against the Venereal Diseases and Treponematoses. All these meetings were attended by British physicians, some of whom contributed papers.

The first professor of genitourinary medicine in the world has recently been appointed at the Middlesex Hospital Medical School, London University, and will take up his appointment on 1 January 1979.

Research into several aspects of the sexually transmitted diseases continues to be carried out at the larger centres and the number and variety of projects is increasing. Support is given by the Medical Research Council and the DHSS and in some instances by other grant-giving bodies. Finance for

Table 4 Other sexually transmitted diseases and other conditions—new cases per 100 000 population at all ages seen at hospital clinics in England 1973-77

Years ending 30 June															
Disease	1973			1974			1975			1976			1977		
	Men	Women	Total	Men	Women	Total	Men	Women	Total	Men	Women	Total	Men	Women	Total
Lymphogranuloma venereum	0·25	0·03*	0·14	0·15	0·03*	0·03	0·18	0·03*	0·10	0·14	0·01*	0·07	0·12	0·04*	0·08
Granuloma inguinale	†	0·01*		0·04	0·02*	0·03	0·02*	0·03*	0·02	0·05	0·02*	0·03	0·26	0·05	0·15
Non-specific genital infection	286·34	60·01	170·10	307·95	63·58	182·50	306·90	65·87	183·25	310·35	73·60	188·91	327·40	84·72	202·95
Non-specific genital infection with arthritis	1·83	0·06	0·92	1·87	0·08	0·95	1·92	0·14	1·01	2·07	0·01	1·06	2·44	0·18	1·28
Trichomoniasis	7·08	72·39	40·02	6·88	74·02	41·35	6·42	74·96	41·58	6·81	76·23	42·42	7·54	76·86	43·09
Candidosis	20·42	108·17	65·49	22·84	115·53	70·42	23·71	116·83	71·48	27·68	125·84	78·02	29·89	125·99	79·18
Scabies	9·23	1·95	5·49	9·64	2·13	5·79	10·20	2·01	6·00	9·41	2·19	5·17	7·82	1·98	4·82
Public lice (pediculosis pubis)	13·50	4·53	8·89	15·37	5·22	10·16	16·07	5·78	10·80	16·07	6·58	11·21	18·06	7·39	12·58
Herpes simplex	14·04	5·56	9·68	15·28	6·88	10·97	16·77	7·86	12·20	19·10	9·83	14·35	20·76	11·08	15·79
Warts (condylomata acuminata)	48·70	25·14	36·60	53·11	26·02	39·20	56·58	27·98	41·91	60·31	30·71	45·13	64·81	32·52	48·25
Molluscum contagiosum	2·15	0·79	1·45	2·02	0·87	1·43	2·26	0·79	1·50	2·53	1·08	1·78	2·72	1·27	1·97
Other treponemal diseases	2·48	1·24	1·84	2·64	1·43	2·02	3·17	1·59	2·36	2·94	1·60	2·25	3·30	1·79	2·53
Other conditions requiring treatment in a centre	103·65	42·02	72·00	112·19	44·55	77·47	111·39	49·70	79·74	112·32	55·75	83·30	114·30	59·38	86·14
Other conditions not requiring treatment in a centre	217·97	115·94	165·57	240·57	132·52	185·10	232·69	132·61	181·35	237·48	136·00	185·43	251·67	145·75	197·35
Other conditions referred elsewhere‡										2·40‡	1·33‡	1·85‡	5·03	3·11	4·05

*Rates based on fewer than 10 events and consequently their reliability as a measure may be affected.

†Indicates that there were no events. #Figures collected from January 1976.

overseas travel for research purposes is also provided by the DHSS.

The potentially very dangerous strains of β -lactamase-producing gonococci, which are totally resistant to penicillin and frequently insensitive to other antibiotics, have not become widespread throughout the country, as at one time was feared. Their incidence is monitored in England by the Public Health Laboratory Service and on a world-wide basis by the WHO. During the year only a small

number of strains were confirmed as β -lactamase producers, most of which were brought into this country from West Africa and the Western Pacific. Nevertheless, these strains could rapidly become widely diffused throughout the population, and constant vigilance and exchange of information is of great importance.

Correspondence

TO THE EDITOR, *British Journal of Venereal Diseases*

Sir,

Pityriasis versicolor of the glans penis

Pityriasis versicolor is a common disease, more so in tropical than in temperate climates.

An Indian man, aged 20, employed as a cook's assistant in the government hospital, attended the dermatology and venereology clinic complaining of loss of pigmentation and scaly patches on his body and tip of his penis. The condition had first developed three years previously with lesions on the upper trunk. He had never taken any treatment but became alarmed when the condition spread progressively and appeared on the genitals, occasionally causing irritation. His general health was good and he gave no past or family history of notable illnesses.

On examination there was a depigmented area on the chest, back, and buttocks formed by coalescence of smaller areas of pityriasis versicolor, which were present elsewhere including the shaft of the penis. The patient had been circumcised in childhood and on the left side of the glans penis there were two circinate, discrete areas of fine scaling, slightly raised, the smaller being on the rim of the glans. These lesions were unlike any other forms of balanitis which we had seen.

Radiation with Wood's light showed yellow fluorescence of some of the lesions on the trunk and all of those on the glans and shaft of the penis. With flashlight photography of the lesions under Wood's light the yellow fluorescence was lost but the glandular lesions showed as areas of scaly white. Scrapings from both trunk and glans gave positive results for spores and mycelium typical of pityriasis versicolor.

The patient was treated with twice-daily application of miconazole cream, and the glandular lesion had resolved to hypopigmentation when he was seen a week later. He did not attend again.

Four hundred cases of pityriasis versicolor were diagnosed out of a total of more than 8000 new referrals in a six-month period, which gives an incidence of approximately 5%. In hot climates involvement of the skin is frequently extensive but the genitals are less commonly affected. Total body examination with Wood's light

is the only means of determining the extent of the disease in dark-skinned patients, and it shows areas of which the patient may be unaware. Five cases of pityriasis versicolor affecting the penile skin were diagnosed among 70 men during a six-month series here. The patient described above had lesions on the skin and on the glans, and this association has not been reported before, even in such detailed studies as that of Roberts (1968) or El-Hefnawi (1971).

It is interesting that pityriasis versicolor does not affect the genitals more, considering the role that heat and moisture are considered to play in its aetiology. On the other hand, it is easy to understand why the surface of the glans is unaffected, because of its different structure.

Yours faithfully,

A. Karim Nia
E. L. Smith

Department of Dermatology,
Government Hospital,
Abu Dhabi,
United Arab Emirates

References

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Roberts, S. O. B. (1968). Pityriasis versicolor: a clinical and mycological investigation. *British Journal of Dermatology*, 81, 315-326.
Smith, E. L. (1978). Pityriasis versicolor of the penis. *British Journal of Venereal Diseases*, 54, 441.

TO THE EDITOR, *British Journal of Venereal Diseases*

Sir,

Anal canal versus rectal wall sampling

Dr R. S. Morton's criticism of my paper (Morton, 1978) has surprised me. It is not that I feel I am beyond criticism, but it is the totally uncalled for manner in which he has done so.

To add insult to injury he has even made incorrect statements. Even the title of my paper has not been correctly interpreted; my paper was entitled 'Diagnosis of rectal gonorrhoea by blind anorectal swabs compared with direct vision swabs taken via a proctoscope'. He has changed this to

'Anal canal versus rectal wall sampling'; it appears to me that he does not understand the word anorectal.

May I point out to Dr Morton once again what I was attempting to prove. The study I did was to compare the results of gonococcal cultures taken from the same site, namely the rectum, using two different methods for obtaining the necessary material. I used the words 'blind anorectal swab' because it is not possible to pass a blind swab into the rectum without first passing it through the anal canal. I concluded from this study that both methods gave satisfactory culture results.

This being the conclusion, what I would like to know from Dr Morton is how more cases of gonococcal proctitis could be diagnosed if a proctoscope had been used. Another misquotation is, 'he reached the conclusion that rectal wall sampling should be confined to the first visits and to patients with symptoms'. What I wrote was, 'It is, however, not suggested that proctoscopic examination should not be done at all but that it could be confined to the first visit and to patients who complain of symptoms'. The word 'should' cannot be substituted for the word 'could' without changing the meaning.

Is he really serious when he says, 'Vaginal sampling is not the best way to diagnose gonococcal endocervicitis'? Is this not obvious? It is certainly not advisable to take a swab from one site to make a diagnosis of an infection at another site; if one does this the results are likely to be unsatisfactory. Despite Dr Morton's implication, this is not what I did.

Finally, it was notable that Dr Morton did not quote any figures to support his argument.

Yours faithfully,

P. Deheragoda

Department of Genitourinary Medicine,
Croydon General Hospital,
London Road,
Croydon,
Surrey CR9 2RH

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- Deheragoda, P. (1977). Diagnosis of rectal gonorrhoea by blind anorectal swabs compared with direct vision swabs taken via a proctoscope. *British Journal of Venereal Diseases*, 53, 311-313.
Morton, R. S. (1978). Anal canal versus rectal wall sampling. *British Journal of Venereal Diseases*, 54, 206.

Book reviews

Basic Sexual Medicine. By Eric Trimmer, 1978. Pp. 238. William Heinemann Medical Books Ltd, London (£6.95).

Sexual medicine has traditionally been imbued with myth and folklore, and hence it is gratifying to find that Dr Trimmer has managed to produce an objective, readable, and very practical book on the subject. The field is extensive, and no doubt the problem was what to leave out rather than how much to put into a fairly slim volume.

The emphasis is rightly on functional sexual disorders. Starting with a description of male and female sex organs, the aetiology and management of sexual dysfunction in men and women is then discussed. Specific regimens, such as masturbation therapy and Sensate Focus techniques, are detailed in an excellent appendix and there are also sections on ageing and sexual function, sex and the handicapped, and intersexuality. The commoner sexual deviations ('paraphilias' is the term preferred by the author) are also dealt with in some detail. Antiandrogenic compounds, such as cyproterone, are advocated to treat paedophiliacs and certain other sexual offenders. Rape and its forensic implications are included in the same chapter. The final section evaluates current trends and assumptions in sexual medicine, and this is the most critical and, in some ways, the best part of the book. The very valid point is made that therapists must collate data on treatment techniques and follow up patients adequately in order to put the management of sexual problems on a more scientific footing.

The text occasionally falters when it encroaches upon the disciplines of gynaecology and venereology. Bartholin's glands are dismissed in a rather cursory fashion as 'probable vestigial structures' and 'medical and surgical treatment' of cervical erosions is mentioned without adequate explanation of these terms. Epididymitis is attributed commonly to weight-lifting with a full bladder and rarely to gonorrhoea or non-specific urethritis; here we are on decidedly uncertain ground.

The clinical and historical notes are appropriate and the work contains many fascinating pieces of information. I did not know for instance that the Devil has a forked penis or that anal intercourse with a woman is still theoretically punishable with

life imprisonment in England and Wales. One certainly has to watch one's step.

J. Davidson Parker

A Textbook of Venereal Diseases. By V. N. Sehgal, 1978. Pp. 117. Vikas Publishing House Pvt Ltd, New Delhi, India (Rupees 36.00).

The book seems to be intended for undergraduate students and general practitioners in Indian sub-continent concerned with the sexually transmitted diseases.

The introductory chapters on epidemiology of venereal diseases and applied anatomy of genital organs are nicely written and are adequate, but there is an important omission. The author has completely ignored the subject of history-taking and examination, which are so important.

The chapters on gonorrhoea and syphilis are well covered, though one may tend to disagree with the author in places, especially concerning treatment.

It was disappointing to find little new on chancroid, a condition rarely seen in the West and so either forgotten or poorly investigated. Chlamydial investigations are not mentioned in the diagnosis of lymphogranuloma venereum. Again, in the diagnosis of granuloma inguinale more practical details could be provided for the taking of tissue smears.

*R. K. Pandhi
R. N. Thin*

The Gonococcus. Edited by Richard B. Roberts, 1977. Pp. 436. John Wiley and Sons Ltd, New York, USA (£24.70).

This is a well-produced and readable book, which deals with all aspects of the gonococcus, although only the first four of the 19 chapters will be of interest to most clinicians. These chapters cover epidemiology, control, diagnostic methods, and clinical aspects. Michael Rein starts with an excellent review of the epidemiology of gonococcal infection, which whets one's appetite for further chapters. It is a shame that the sections devoted to control and prevention of gonorrhoea in the USA and UK are written in such disparate styles, making comparison difficult. Most of the UK data

comes from the British Co-operative Clinical Group Survey of 1970, which is rather dated now. Overall, there is a strong North American bias with only three of the 29 contributors working in Europe. Treatment regimens are, therefore, sometimes excessive by UK standards (for example, 20 megaunits crystalline penicillin G daily for pelvic infection), and no argument is advanced against epidemiological treatment. Luckily, few neologisms are found, although Londoners are presumably part of 'the urbanised'. It is good to see Douglas Kellogg suggesting rapid methods for recognising the presence of gonococci.

There are useful reviews of auxotyping, plasmids, pili, and interactions of gonococci with epithelial cells and leucocytes, and chapters on gonococcal biochemistry and physiology. Unfortunately, the emergence of the β -lactamase-producing gonococci predicted by Dr Falkow came rather late in the preparation of this book and, therefore, a relatively brief mention of the problem is made, which is a pity. Recent work on other forms of antibiotic resistance, however, are well covered. It is interesting to learn that the gonococcus has a restricted metabolic potential, providing further evidence that this organism is highly adapted to its host. There are a few typographical errors. The index is adequate, and most chapters have a useful list of references. This book competes favourably with others recently published on the same subject, but it is expensive.

*R. N. Thin
P. D. Simmons*

Dermatology and Venereology (Dermatologie und Venereologie). By A. Greither, 1978. Pp. 234, 82 figs. Heidelberg Taschenbücher Basistext Medizin, third edition, vol. 113. Springer, Berlin.

The first edition of this excellent textbook for medical students was reviewed by G. W. Csonka (1973), and his criticisms of its shortcomings in the field of genitourinary medicine remain valid.

The section on venereology remains short, mentioning only gonorrhoea, chancroid, lymphogranuloma venereum, granuloma inguinale, and syphilis. Teaching on these diseases is didactic, but

perhaps that is what a student requires. Not all would agree on this side of the Channel that Reiter's syndrome is a complication of gonorrhoea. It is interesting to note German regimens for penicillin treatment in syphilis; they are much longer than those in use in this country.

There is still no mention of non-gonococcal urethritis. A diligent search will find sections on trichomoniasis, candidosis

(no mention of the tritylimidazole derivatives), scabies, pediculosis, herpes genitalis, condylomata acuminata, and molluscum contagiosum arranged in appropriate taxonomic groups. Concise descriptions of the disease, differential diagnosis, and treatment are given. One criticism is that drugs are often mentioned only by their German proprietary names. Together with many of the descriptions of

disease, there are excellent summaries of the history of discovery of the disease.

The last chapter is on andrology, a novel partner for dermatology and venereology.

M. A. Waugh

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Csonka, G. W. (1973). *British Journal of Venereal Diseases*, 49, 395.

Notices

Dutch Society for the Study of STDs

On 14 December, 1978, the Dutch Society for the Study of Sexually Transmitted Diseases was founded at Rotterdam. The membership of this society is open to medical and non-medical persons of all disciplines who are interested in or working in the field of the study of sexually transmitted diseases. Scientific sessions will be held twice a year.

The secretary is Dr H. E. Menke, Department of Dermatology and Venereology, University Hospital, Rotterdam, the Netherlands.

African Branch of the IUVDT

The African Branch of the International Union against the Venereal Diseases and Treponematoses has been formed and the Inaugural Meeting will be held from 23 to 25 August, 1979, at the Conference Centre, University of Ibadan, Nigeria.

Further details may be obtained from Dr A. Osoba, Department of Microbiology, University College Hospital, Ibadan, Nigeria.

Abstracts

These selected abstracts and titles from the world literature are arranged in the following sections:

Syphilis and other treponematoses

(Clinical and therapy; serology and biological false-positive phenomenon; pathology and experimental)

Gonorrhoea

(Clinical; microbiology; therapy)

Non-specific genital infection

Reiter's disease

Trichomoniasis

Candidosis

Genital herpes

Other sexually transmitted diseases

Public health and social aspects

Miscellaneous

Syphilis and other treponematoses (Clinical and therapy)

Treatment of early latent syphilis of less than a year's duration: an evaluation of 275 cases

N. J. FIUMARA (1978). *Sexually Transmitted Diseases*, 5, 85-88

Syphilis (Serology and biological false-positive phenomenon)

Comparison of a haemagglutination treponemal test for syphilis (HATTS) with other serologic methods for the diagnosis of syphilis

B. B. WENTWORTH, M. S. THOMPSON, C. R. PETER, R. E. BAWDON, AND D. L. WILSON (1978). *Sexually Transmitted Diseases*, 5, 103-111

Three laboratories participated in studies of a haemagglutination treponemal test for syphilis (HATTS), using as antigen sonicated *Treponema pallidum* coupled to glutaraldehyde-stabilised turkey erythrocytes by the bis-diazotized benzidine procedure. A total of 1056 cases of syphilis (373 untreated and 683 treated) was studied, with 93.7% overall agreement between the HATTS and the fluorescent treponemal antibody absorption (FTA-ABS) test. In addition, 1805 non-syphilitic sera, including 1048 from presumably healthy subjects, 502 sera that were biological false-positives, and 255 sera from

patients with diseases other than syphilis, were studied. The HATTS was non-reactive for 99.4% of sera from presumably healthy subjects, 86.9% of biological false-positive sera, and 84.7% of sera from patients with diseases other than syphilis compared with FTA-ABS non-reactivity for 99.2% of presumably normal sera, 76.9% of false-positive sera, and 77.3% of sera from patients with other diseases. One laboratory also compared the HATTS with the micro-haemagglutination—*Treponema pallidum* (MHA-TP) test. There was no statistically significant ($P < 0.01$) difference in reactivities among the HATTS, MHA-TP, and FTA-ABS procedures for either 617 syphilitic or 966 non-syphilitic sera. Studies of reproducibility showed no significant difference in performances of the HATTS by three laboratories, and the HATTS was significantly more reproducible than the MHA-TP test. It is concluded that the HATTS would be a suitable substitute for the MHA-TP or FTA-ABS test as a confirmatory test for the diagnosis of syphilis.

Authors' summary

Sierologia della sifilide: ELISA, un test di terza generazione (Serology of syphilis: ELISA, a third generation test)

G. SACCHI, E. ALESSI, G. VALCURONE, C. BUZZETTI, AND G. C. TASSI (1978). *Bollettino dell'Istituto Sieroterapico Milanese*, 57, 490-515

A purified protein extracted from the cultivable Reiter treponeme was used as antigen and reactivity of sera with this detected by the addition of anti-human IgG

conjugated with peroxidase; o-phenylenediamine was used as the substrate. Methods for preparation and standardisation of the reagents are described. At the end of the test the optical density (OD) of the test sample at 490 nm was divided by the mean plus standard error of the OD of 10 negative control sera and values greater than one taken as positive.

The ELISA technique was compared with the WR, RPCFT, VDRL, FTA-ABS, TPI, and TPHA tests on 295 sera from patients with untreated or treated early syphilis, 120 sera from blood donors who had no history, signs, or serological evidence of syphilis, and on 20 sera which had given false-positive (BFP) reactions with tests for reagin or the RPCFT. The ELISA test gave positive results in 98.47% of the untreated primary, 100% of the untreated secondary, and 95% of the treated early cases and was more sensitive than any of the other tests performed. It gave negative results with all of the BFP reactors and with the sera from normal donors (although this is not very clear from the text).

Because of its high sensitivity and specificity, ease of execution and the objective method of reading, the test is thought to be of potential value for the serodiagnosis of syphilis.

A. E. Wilkinson

(Reprinted from *Abstracts on Hygiene* by permission of the Editor.)

Statistical aspects of the treponemal counts in the TPI test

S. CHRISTIANSEN (1978). *Acta Pathologica et Microbiologica Scandinavica*, 86, 267-274

Evaluation of the quantitative Rappaport rapid plate test in the diagnosis and treatment of syphilis

R. GHINSBERG, E. MEIR, G. BLUMSTEIN, AND R. KAFMAN (1978). *Israel Journal of Medical Sciences*, **14**, 1079-1081

Syphilis (Pathology and experimental)

Transplacental transmission of spirochaetes in congenital syphilis: a new perspective (leading article)

W. D. HAGER (1978). *Sexually Transmitted Diseases*, **5**, 122-123

Influence of oxygen tension, sulphhydryl compounds and serum on the motility and virulence of *Treponema pallidum* (Nichols strain) in a cell-free system

S. J. NORRIS, J. N. MILLER, J. A. SYKES, AND T. J. FITZGERALD (1978). *Infection and Immunity*, **22**, 689-697

Genetics of *Treponema*: characterisation of *Treponema hyodysenteriae* and its relationship to *Treponema pallidum*

R. M. MIAO, A. H. FIELDSTEEL, AND D. L. HARRIS (1978). *Infection and Immunity*, **22**, 736-739

Gonorrhoea (Clinical)

Gonorrhoea in pregnancy

L. E. EDWARDS, M. I. BARRADA, A. A. HAMANN, AND E. Y. HAKANSON (1978). *American Journal of Obstetrics and Gynecology*, **132**, 637-641

Retrospective examination was made of the records of 6464 women delivered of their fetuses over an eight-year period to define the effects of antepartum and intrapartum gonococcal infection on the course and outcome of pregnancy. Each patient with gonorrhoea was matched with between two and four control patients for age, race, parity, socioeconomic status, and time of delivery.

A total of 178 (8%) women had at least one positive culture result during their pregnancy though only 19 had positive results at delivery. Disseminated

gonococcal infection was found in 0.03% of all pregnant women. Chorioamnionitis and intrauterine growth retardation occurred more frequently in a significant ($P < 0.05$) proportion of women with positive culture results at some time during pregnancy. When compared with controls patients with an intrapartum infection had a significant increase in premature rupture of membranes ($P < 0.05$ —any duration, $P < 0.02$ —less than 24 hours), chorioamnionitis ($P < 0.05$), premature infants ($P < 0.02$) and infants with positive orogastric culture results ($P < 0.01$).

In conclusion, the possibility that intrauterine fetal infection may develop in the absence of prematurely ruptured membranes or clinically evident chorioamnionitis was raised.

R. S. Pattman

ESR in gonococcal arthritis

D. MURRAY (1979). *British Medical Journal*, **1**, 22-23

Gonococcal ophthalmia neonatorum caused by beta-lactamase-producing *Neisseria gonorrhoeae*

R. PANG, L. B. TEH, V. S. RAJAN, AND E. H. SNG (1979). *British Medical Journal*, **1**, 380

Gonorrhoea (Microbiology)

Enzyme-linked immunosorbent assays for the detection of *Neisseria gonorrhoeae* specific antibodies

B. R. BRODEUR, F. E. ASHTON, AND B. B. DIENA (1978). *Canadian Journal of Microbiology*, **24**, 1300-1305

Conditions for the use of enzyme-linked immunosorbent assay (ELISA) in detecting antibody against outer membrane protein complex (OMC) isolated from *Neisseria gonorrhoeae* were investigated using antigenococcal serum raised in rabbits against formalinised whole cells. Optimal binding concentrations of OMC antigen, primary antibody, and alkaline phosphatase conjugated secondary antibody were studied. Antigen solution containing 10 µg/ml protein was found to give maximum coating of the wells of the microtitre plate used in the assay. Incubation for one hour at 37°C or for 18

hours at ambient temperature resulted in similar coating: for convenience the latter conditions were adopted. Optimal binding of the primary antibody and enzyme-conjugated anti-immunoglobulin was achieved after one hour at 37°C. A positive reaction (absorbance value of less than 0.15 at 400 nm) was obtained on testing OMC antigen prepared from five different gonococcal strains against a single antiserum prepared from one of the strains. Pre-incubation of antiserum with homologous OMC antigen inhibited the subsequent immunological reaction: using this technique it was possible to measure levels of antigen as low as 0.5 µg/ml. Gonococcal antisera showed no significant cross-reaction with OMC antigen prepared from six strains of *Neisseria meningitidis* and from seven strains of non-pathogenic *neisseriae*. (Antisera prepared against other *neisseriae* were not tested against gonococcal OMC antigen.)

H. Young

Rapid micro-carbohydrate test for confirmation of *Neisseria gonorrhoeae*

D. C. T. YONG AND A. PRYTULA (1978). *Journal of Clinical Microbiology*, **8**, 643-647

The rapid carbohydrate utilisation procedure described uses both preformed enzymes and enzymes formed by the micro-organisms as a result of growth in a small volume of superenriched medium containing the appropriate carbohydrate and a pH indicator. When a loopful of bacteria subcultured once from the primary isolation medium and grown for 18 to 24 hours on chocolate agar was used as inoculum carbohydrate utilisation reactions were completed within four hours of incubation at 36°C. All 383 clinical isolates of *neisseriae* (377 strains of gonococci and six strains of meningococci) tested gave the expected reaction in the rapid test whereas only 358 of the gonococcal strains and four of the meningococcal strains gave the expected reaction with the conventional cystine-trypticase agar method.

The authors discuss the limitations of existing carbohydrate utilisation techniques and point out that the combined action of two sources of enzyme (preformed and formed by growth during the test) in their method is an advantage since this produces results which are not affected by small variations in either the inoculum size or growth requirements of individual strains. The same principle endows the test with a

disadvantage in that it is unlikely that it could be used to characterise isolates directly from primary isolation cultures because of interference by small numbers of contaminating organisms.

H. Young

Antigen-specific serotyping of *Neisseria gonorrhoeae*: I Use of an enzyme-linked immunosorbent assay to quantitate pilus antigens on gonococci

T. M. BUCHANAN (1978). *Journal of Infectious Diseases*, 138, 319-325

Purified pili from *Neisseria gonorrhoeae* were used in an enzyme-linked immunosorbent assay (ELISA) to quantitate human or rabbit antibodies to pili; amounts of pilus antigen on different gonococci were quantitated, and yields of pili during purification were determined in ELISA by the degree of inhibition of optical density. The amount of pilus antigen expressed on the surface of colony type 1 or 2 gonococci of three different strains varied from 450 to 9000 ng/600 μ l of a 200-Klett unit suspension. The quantity of pilus antigen was correlated directly with the extent of piliation as determined by electron microscopy. No pilus antigen was found by ELISA in colony type 4 organisms (devoid of pili) of three different strains. No more than 10% shared antigenicity was observed for antigenically different pili. Present purification procedures for gonococcal pili provide a yield of 15%. ELISA may allow better evaluation and quantitation of the potential roles of antibody to pili in the killing or opsonisation of gonococci or in the inhibition of gonococcal attachment to human cells.

Author's summary

Studies on gonococcus infection. XVI Purification of *Neisseria gonorrhoeae* immunoglobulin A₁ protease

M. S. BLAKE AND J. SWANSON (1978). *Infection and Immunity*, 22, 350-358

A protease which cleaves human immunoglobulin A₁ (IgA₁) has been purified from broth cultures of *Neisseria gonorrhoeae*. This IgA₁ protease is produced by pilated and non-piliated gonococci throughout their growth cycles. A combination of ammonium sulphate precipitation, column chromatography, and either isoelectric focusing or affinity chromatography was used to obtain an enzyme preparation that showed approximately 3800-fold purifi-

cation and exhibited two bands (65 000 and 70 000 daltons) by analytical polyacrylamide electrophoresis in the presence of sodium dodecyl sulphate and reducing conditions. IgA₁ protease activity is dependent on divalent cations and is heat labile. Detection and quantitation of IgA protease activity used an assay in which (I¹²⁵) IgA₁ is incubated with protease preparations and the cleavage products are analysed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis.

Authors' summary

Comparison of antigenic heterogeneity of *Neisseria gonorrhoeae* strains by micro-immunofluorescence and serum bactericidal tests

J. A. MARK AND S.-P. WANG (1978). *Infection and Immunity*, 22, 403-410

The antigenic heterogeneity of *Neisseria gonorrhoeae* strains was assessed by the micro-immunofluorescence (micro-IF) and the serum bactericidal tests. The micro-IF test verified the antigenic heterogeneity of nine strains received from the Centre for Disease Control and placed them into immunotypes A and B. The serum bactericidal system also detected different antigenic determinants among the strains. Although the micro-IF and bactericidal assays did not correspond in each instance, the overall pattern of similarities and differences among these gonococcal strains was similar. The micro-IF pattern obtained with mouse antisera was identical to that obtained with guinea pig antisera. Different colony-type organisms showed similar sensitivity in the bactericidal test. The micro-IF test is a rapid technique for the immunotyping of *N. gonorrhoeae* and has the additional advantages of reproducibility and simplicity.

Authors' summary

***Neisseria* confirmation by an enriched, bicarbonate-containing carbohydrate medium**

J. O. GRAVES AND L. A. MAQEE (1978). *Journal of Clinical Microbiology*, 8, 525-528

A sugar fermentation medium for the confirmation of *Neisseria* and related species was developed. The medium contained a commercial supplement and a haemoglobin source prepared from lysed sheep erythrocytes. Bicarbonate in the medium substituted for a CO₂-supple-

mented atmosphere. The medium was dispensed into screw-capped tubes. This medium was compared to cystine-trypticase agar and the modified rapid fermentation test in the confirmation of *Neisseria* species. Performance of the new medium was equivalent to that of the modified rapid fermentation test, but cystine-trypticase agar failed to confirm a significant number of clinical isolates of *Neisseria gonorrhoeae*.

Authors' summary

A simple manganous chloride and Congo-red disc method for differentiating *Neisseria gonorrhoeae* from *Neisseria meningitidis*

T. O. ODUGBEMI, M. G. MCENTEGART, AND S. HAFIZ (1978). *Journal of Clinical Pathology*, 31, 936-938

Manganous chloride and Congo-red incorporated into blotting-paper discs have been used to differentiate gonococci from meningococci. The new technique is simple and reliable; the materials for the test are inexpensive. The method will increase the efficiency of distinguishing between the pathogenic *Neisseria* in any clinical bacteriology laboratory and especially in those in the tropical areas.

Authors' summary

Binding of cholesterol by *Neisseria gonorrhoeae*

R. D. MILLER, W. J. WARREN, R. C. SIZEMORE, AND S. A. MORSE (1978). *Infection and Immunity*, 22, 698-708

Effects of thiamphenicol and chloramphenicol in inhibiting *Neisseria gonorrhoeae* isolates

P. D. DUCK, J. R. DILLON, AND L. EIDUS (1978). *Antimicrobial Agents and Chemotherapy*, 14, 788-790

Inhibition of β -lactamase in *Neisseria gonorrhoeae* by sodium clavulanate

J. M. MILLER, C. N. BAKER, AND C. THORNSBERRY (1978). *Antimicrobial Agents and Chemotherapy*, 14, 794-796

Gonorrhoea (Therapy)

A comparison of the in vitro activity of rosamicin, erythromycin, penicillin and tetracycline against *N. gonorrhoeae*, including β -lactamase producing isolates

J. R. DILLON, P. D. DUCK, AND L. EIDUS (1978). *Journal of Antimicrobial Chemotherapy*, 4, 477-479

This paper examines the in-vitro activity of rosamicin, erythromycin, spiramycin, penicillin, and tetracycline against 517 non-penicillinase-producing strains, and 13 penicillinase-producing strains of *N. gonorrhoeae*. Susceptibility testing was carried out using a standard agar plate dilution method. At a level of 0.1 $\mu\text{g/ml}$ rosamicin inhibited 90.9% of the non-penicillinase-producing strains while a range of 25.7% to 56.3% was obtained with the other antibiotics with the exception of spiramycin. Spiramycin was the least active, even at a level of 4 $\mu\text{g/ml}$; only 66.3% of these isolates were inhibited. Rosamicin inhibited 46.2% of the penicillinase-producers at 0.1 $\mu\text{g/ml}$, compared with 30.8% with erythromycin.

These results indicate that rosamicin may prove useful in the therapy of gonorrhoea due to both non-penicillinase-producing and penicillinase-producing strains of *N. gonorrhoeae*, indicating a need for clinical studies.

G. L. Ridgway

Therapy of gonorrhoea. Comparison of trimethoprim-sulfamethoxazole and ampicillin

F. R. SATTler AND J. RUSKIN (1978). *Journal of the American Medical Association*, 240, 2267-2270

Eighty-nine men with gonococcal urethritis were randomly treated with trimethoprim-sulphamethoxazole, four tablets (trimethoprim 320 mg and sulphamethoxazole 1600 mg) twice daily for two days, or ampicillin 3.5 g plus probenecid 1 g in a single dose. Forty-one (95.3%) of 43 patients who received trimethoprim-sulphamethoxazole and 41 (97.6%) of 42 given ampicillin were cured. Neither drug caused major side effects. All isolates of *Neisseria gonorrhoeae* were susceptible in vitro to trimethoprim-sulphamethoxazole and all but one were inhibited by ampicillin. The ampicillin-resistant strain (MIC=4 $\mu\text{g/ml}$) produced penicillinase and was recovered from a patient who

responded to treatment with trimethoprim-sulphamethoxazole. There was no significant correlation between the MICs of trimethoprim-sulphamethoxazole and ampicillin. It is concluded that trimethoprim-sulphamethoxazole is as efficacious and safe as ampicillin in the therapy of gonococcal urethritis.

Authors' summary

A single large dose of trimethoprim-sulfamethoxazole fails to cure gonococcal urethritis in men

R. B. PRIOR, R. J. FASSE, AND R. L. PERKINS (1978). *Sexually Transmitted Diseases*, 5, 62-64

In a single-blind study, 50 men who had acute gonococcal urethritis were treated with a single oral dose of either 720 mg trimethoprim (TMP) plus 3600 mg sulphamethoxazole (SMZ) or 3.5 g ampicillin plus 1 g probenecid. Isolates of *Neisseria gonorrhoeae* were tested for in-vitro susceptibility to the chemotherapeutic agents administered by agar-dilution and disc-diffusion methods, and results were correlated with cure or failure to cure as determined bacteriologically. Among patients returning for follow up, the cure rate after TMP/SMZ was 69%. Cure was predictable when the isolates of *N. gonorrhoeae* were inhibited by $\leq 0.63/11.87 \mu\text{g/ml}$ of TMP/SMZ (fixed ratio, 1:19) or when the zones of inhibition were ≥ 23 mm; failure was predictable when $\geq 1.25/23.75 \mu\text{g/ml}$ of TMP/SMZ was necessary for inhibition and when zones of inhibition were ≤ 21 mm ($P < 0.02$). The cure rate after therapy with ampicillin was 100%, a rate significantly higher than that found after TMP/SMZ ($P < 0.02$); all isolates were inhibited by $\leq 0.16 \mu\text{g/ml}$ of ampicillin. Adverse reactions were not seen with either TMP/SMZ or ampicillin.

Authors' summary

A rational basis for the epidemiologic treatment of gonorrhea in a clinic for sexually transmitted diseases

F. N. JUDSON AND A. B. MALTZ (1978). *Sexually Transmitted Diseases*, 5, 89-92

Epidemiological treatment of gonorrhoea refers to the administration of antibiotics when the diagnosis is considered likely but before the results of confirmatory tests are available. Unfortunately, the risk of infection is seldom known. To place epidemiological treatment on a more

rational basis, infection rates were determined for groups of clinic patients defined by easily collected indexes of risk, such as reason for attending the clinic, sex, race, sexual preference, history of contact with gonorrhoea, and history of a urethral or vaginal discharge. Infection rates ranged from 0.8% for men seeking marriage licences to 65.1% for female contacts of men with 'established' gonorrhoea. By selectively employing Gram-stained smears, it is possible to reduce further the need for epidemiological treatment within the various defined groups of patients. Epidemiological treatment policies should be determined by each large clinic and should be based on known infection rates for clearly defined groups of patients. Such rates are necessary for obtaining informed consent from patients and for evaluation of the cost-effectiveness of epidemiological treatment in the overall effort to control gonorrhoea.

Authors' summary

Epidemiologic treatment of gonorrhea (leading article)

P. J. WIESNER (1978). *Sexually Transmitted Diseases*, 5, 120-121

Tracing and treating contacts of gonorrhea patients in a clinic for sexually transmitted diseases

F. N. JUDSON AND F. C. WOLF (1978). *Public Health Reports*, 93, 460-463

Patient variables associated with penicillin resistance in *Neisseria gonorrhoeae*

C. THORNSBERRY, H. W. JAFFE, G. H. REYNOLDS, A. A. ZAIDI, AND P. J. WIESNER (1978). *Antimicrobial Agents and Chemotherapy*, 14, 327-330

Non-specific genital infection

Examination of men with nongonococcal urethritis and their sexual partners for *Chlamydia trachomatis* and *Ureaplasma urealyticum*

J. PAAVONEN, M. KOUASA, P. SAIKKU, E. VESTERINEN, E. JANSSON, AND A. LASSUS (1978). *Sexually Transmitted Diseases*, 5, 93-96

Chlamydia trachomatis was recovered from 39 (52%) of 75 men who had non-

gonococcal urethritis and from 28 (37%) of their sexual partners. Of the partners of men with chlamydiae-positive nongonococcal urethritis, 64% excreted chlamydiae compared with 8% of the partners of men with chlamydiae-negative nongonococcal urethritis. In contrast, an apparently sexual mode of transmission was not observed with *Ureaplasma urealyticum*. Rates of recovery of *U. urealyticum* from men with nongonococcal urethritis whose cultures were chlamydiae-positive and those whose cultures were chlamydiae-negative were the same. Significant seroconversion was detected by the single-antigen immunofluorescence test in about 50% of patients who had chlamydiae-positive cultures.

Authors' summary

Fifteen-month follow-up study of women infected with *Chlamydia trachomatis*

W. M. McCORMACK, S. ALPERT, D. E. McCOMB, R. L. NICHOLS, D. Z. SEMINE, AND S. H. ZINNER (1979). *New England Journal of Medicine*, 300, 123-125

An unselected group of women attending the gynaecologist in a university health service was studied for evidence of chlamydial infection. *C. trachomatis* was isolated from the cervix from 20 (4.6%) of 439 women examined during 1974-75. Local antibody was detected by an indirect immunofluorescence method in 60 (13%) of 463 women, including all but two of those whose cultures yielded *C. trachomatis*. During 1976, follow-up examinations were performed on 25 women whose genital secretions had contained *C. trachomatis*, chlamydial antibody, or both during 1974-75. Eleven women had taken antimicrobial agents active against chlamydiae in the interim and none of these were found to be infected in 1976. Seven of the remaining 14 women were found to be infected 16-17 months after their initial examination. This group included four of seven women from whom *C. trachomatis* was isolated during the initial examination and three of seven whose genital secretions had contained chlamydial antibody but not *C. trachomatis*. The contact histories obtained from these women did not indicate that reinfection was a likely cause of their prolonged chlamydial infection.

J. D. Oriel

Serological typing of *Ureaplasma urealyticum* isolates from urethritis patients by an agar growth inhibition method

M. C. SHEPARD AND C. D. LUNCEFORD (1978). *Journal of Clinical Microbiology*, 8, 566

A method of dividing *Ureaplasma urealyticum* into eight serological types by an agar-growth inhibition is described in detail, and the results of serotyping 338 isolates given.

Of 122 isolates from military personnel with NGU the predominating serotype was type 4 (52%). This serotype was also the commonest isolate from patients (both men and women) with other disorders of the genitourinary tract. Among asymptomatic carriers the commonest serotype was type 8 (30%) but the next most frequently isolated was type 4 (24%).

M. C. Kelsey

A comparison of genital infections caused by *Chlamydia trachomatis* and by *Neisseria gonorrhoeae*

J. T. SMITH, L. A. WEED, G. R. PETTERSEN, AND P. C. O'BRIEN (1978). *American Journal of Clinical Pathology*, 70, 333-336

The potential for vaccine against infection of the genital tract with *Chlamydia trachomatis* (review article)

J. T. GRAYSTON AND S.-P. WANG (1978). *Sexually Transmitted Diseases*, 5, 73-77

Genital mycoplasma infections

J. FRIBERG (1978). *American Journal of Obstetrics and Gynecology*, 132, 573-578

Cell fractions and enzymatic activities of *Ureaplasma urealyticum*

N. ROMANO AND R. L. LICATA (1978). *Journal of Bacteriology*, 136, 833-843

Reiter's disease

Frequent association of *Chlamydia* infection with Reiter's syndrome

M. KOUSA, P. SAIKKU, S. RICHMOND, AND A. LASSUS (1978). *Sexually Transmitted Diseases*, 5, 57-61

The incidence of infection with *Chlamydia trachomatis* in 113 men with Reiter's

syndrome was investigated. Chlamydiae were isolated from urethral specimens from 40 (39%) of 103 patients and from 36 (69%) of 52 of these men who had signs of urogenital inflammation at the time of examination. Chlamydial antibodies (titres, ≥ 8) were detected in sera from 66 (63%) of 104 patients by the complement-fixation test and in sera from 79 (87%) of 91 by a single-antigen indirect immunofluorescence test. Fluorescent chlamydial antibodies were found with equal frequencies in sera from patients whose cultures were negative and sera from patients whose cultures were positive, but the geometric mean titre was higher for the latter group. The results suggest that Reiter's syndrome is frequently associated with cultural or serological evidence of genital infection with *C. trachomatis* or both.

Authors' summary

Trichomoniasis

Evaluation of the indirect hemagglutination technique for study of *Trichomonas vaginalis* infections, particularly in men

T. KUBERSKI (1978). *Sexually Transmitted Diseases*, 5, 97-102

The indirect haemagglutination (IHA) technique was evaluated for use in the serological study of infection with *Trichomonas vaginalis*. The IHA test showed that sera from 88% of women attending a venereal disease clinic had antibody to *T. vaginalis*. The antibody frequency and titres were highest in women who had documented infections due to *T. vaginalis*. Serological and cultural evidence of recent or active trichomonal infection was found in 11% of 85 men who had nongonococcal urethritis but was absent in a control group of 27 men. Clinical findings in 10 men with symptomatic genitourinary trichomoniasis are described; all but one had relatively high ($\geq 1/80$) IHA antibody titres. Antibody to *T. vaginalis* was found significantly ($P < 0.005$) more often in sera from women than in sera from men in an apparently healthy group of individuals between the ages of 1 and 20 years. The IHA test appears potentially useful for the diagnosis of trichomoniasis in men and in the sero-epidemiology of infections due to *T. vaginalis*.

Author's summary

Single-dose metronidazole for trichomonal vaginitis—patient and consort

J. R. DYKERS (1978). *American Journal of Obstetrics and Gynecology*, **132**, 579

Trichomoniasis treated with a single dose of benzylmetronidazole

R. F. ROOS (1978). *South African Medical Journal*, **54**, 867-870

Candidosis**In vitro studies of amphotericin B in combination with the imidazole antifungal compounds clotrimazole and miconazole**

R. F. COSGROVE, A. E. BEEZER, AND R. J. MILES (1978). *Journal of Infectious Diseases*, **138**, 681-685

The clinically important polyene antibiotic amphotericin B in combination with two antifungal imidazole compounds, clotrimazole and miconazole, was studied in vitro. With use of results of cytoplasmic leakage, metabolic heat output, and minimal inhibitory concentration studies a definite antagonistic response was demonstrated. It is suggested that, if combined antifungal drug therapy is clinically indicated, the drug combination should be tested against the isolate by the simple technique of measuring cytoplasmic leakage or by the more elaborate method of flow microcalorimetry.

Authors' summary

Cell-mediated immune deficiency and heightened humoral immune response in chronic vaginal candidiasis

S. MATHUR, J. M. GOUST, E. O. HORGER III, AND H. H. FUDENBERG (1978). *Journal of Clinical and Laboratory Immunology*, **1**, 129-134

Genital herpes**Comparison of various macrophage-inhibitory agents on vaginal and systemic herpes simplex virus type 2 infections**

N. B. McGEORGE AND P. S. MORAHAN (1978). *Infection and Immunity*, **22**, 623-630

Suppression of in vitro growth of virulent and avirulent herpes simplex viruses by cell-mediated immune mechanisms, antibody, and interferon

F. SHIMIZU, J. SATOH, M. TADA, AND K. KUMAGAI (1978). *Infection and Immunity*, **22**, 752-756

Protection of guinea pigs against primary and recurrent genital herpes infections by immunization with live heterologous or homologous herpes simplex virus: implications for a herpes virus vaccine

M. SCRIBA (1978). *Medical Microbiology and Immunology*, **166**, 63-70

Other sexually transmitted diseases**5-fluorouracil urethral suppositories for the eradication of condyloma acuminata**

G. W. WEIMAR, L. A. MILLEMAN, T. L. REILAND, AND D. A. CULP (1978). *Journal of Urology*, **120**, 174-175

Miscellaneous**Use of enteric vaccines in protection against chlamydial infections of the genital tract and the eye of guinea pigs**

R. L. NICHOLS, E. S. MURRAY, AND P. E. NISSON (1978). *Journal of Infectious Diseases*, **138**, 742-745

Guinea pigs in a test group were fed live guinea pig inclusion conjunctivitis (GPIC) organisms classified as *Chlamydia psittaci* in 60% yolk-sac suspensions as enteric vaccines while animals in a control group received uninfected yolk sac. Seven test animals and 14 control animals were challenged 11 or 22 days later with 100 50% infectious doses of GPIC organisms in either the conjunctiva or the vagina. Evidence of protection from mucosa infection in both sites was noted in test animals. Clinically, the disease was less severe and, microbiologically, lower percentages of mucosal cells were infected. The results suggest that enteric vaccination against mucosal infections of the eye and the genital tract with chlamydial agents is possible.

Authors' summary

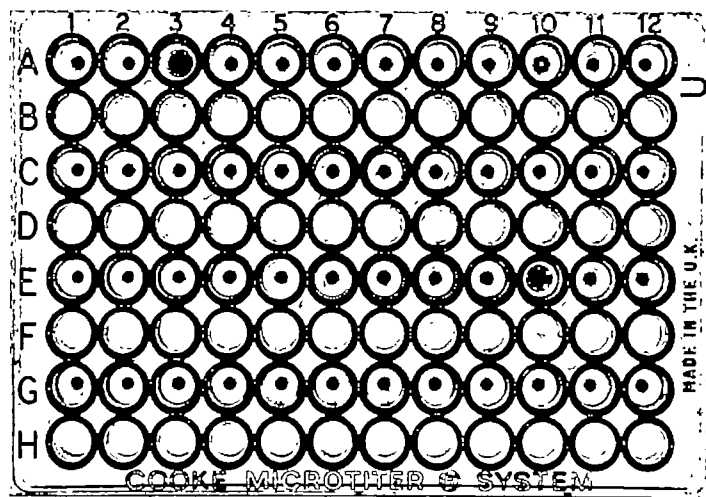
Pigmented penile papules with carcinoma in-situ changes

H. I. KATZ, Z. POSALAKY, AND D. MCGINLEY (1978). *British Journal of Dermatology*, **99**, 155-162

A family study of Behcet's syndrome

M. A. CHAMBERLAIN (1978). *Annals of the Rheumatic Diseases*, **37**, 459-465

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Contents

The Harrison Lecture, 1978: Sexually transmitted diseases and travel

C. S. NICOL (Gt. Britain) *page 161*

Ultrastructural study of satellite lymph nodes in syphilitic rabbits

R. W. LAPUSHIN, R. E. BAUGHN, D. M. MUSHER, AND P. GYORKEY (USA) *page 168*

Treatment monitoring in syphilis using the autoanalyser

D. E. MacFARLANE AND A. McMILLAN (Gt Britain) *page 173*

Rapid presumptive diagnosis of gonococcal urethritis in men by the limulus lysate test

V. A. SPAGNA, R. B. PRIOR, AND R. L. PERKINS (USA) *page 179*

Assessment of a selective medium for the isolation of *Neisseria gonorrhoeae*

E. TAYLOR AND I. PHILLIPS (Gt Britain) *page 183*

Comparison of anal and rectal swabs in the diagnosis of anorectal gonorrhoea in women

B. KOLATOR AND P. RODIN (Gt Britain) *page 186*

Individual susceptibility to neisserial infection?

H. YOUNG, A. B. HARRIS, AND D. H. H. ROBERTSON (Gt Britain) *page 188*

Mezlocillin in the treatment of gonorrhoea

A. LASSUS AND O-V. RENKONEN (Finland) *page 191*

Growth and effect of chlamydiae in human and bovine oviduct organ cultures

G. R. HUTCHINSON, D. TAYLOR-ROBINSON, AND R. R. DOURMASHKIN (Gt Britain) *page 194*

Chlamydia trachomatis in acute salpingitis

J. PAAVONEN, P. SAIKKU, E. VESTERINEN, AND K. AHO (Finland) *page 203*

HLA and sexually transmitted diseases in prostitutes

S. H. CHAN, T. TAN, A. KAMARUDIN, G. B. WEE, AND V. S. RAJAN (Singapore) *page 207*

Clinical pharmacological studies of amoxycillin: effect of probenecid

R. BARBHAIYA, R. N. THIN, P. TURNER, AND J. WADSWORTH (Gt Britain) *page 211*

Haematuria presenting in outpatients attending a department of genitourinary medicine

K. L. AMARASURIYA (Gt Britain) *page 214*

Seasonal variations in the reported incidence of sexually transmitted diseases in Scotland (1972-1976)

C. B. S. SCHOFIELD (Gt Britain) *page 218*

Reinfection with disseminated gonococcal disease: a case report

N. J. FIUMARA AND A. D. SIMKIN (USA) *page 223*

Sexually transmitted diseases: Extract from the Report of the Chief Medical Officer of the Department of Health and Social Security, 1977 *page 225*

Correspondence *page 230*

Book reviews *page 231*

Notices *page 232*

Abstracts *page 233*

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¹Thin RN, Shaw EJ. Diagnosis of gonorrhoea in women. *Br J Vener Dis* 1979; 55: 224-225.

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²King A, Nicol CS. *Venereal Diseases*. 3rd ed. London: Baillière and Tindall, 1975.

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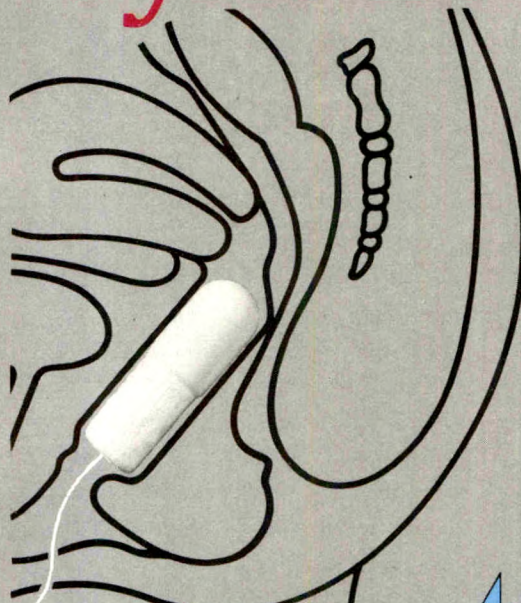
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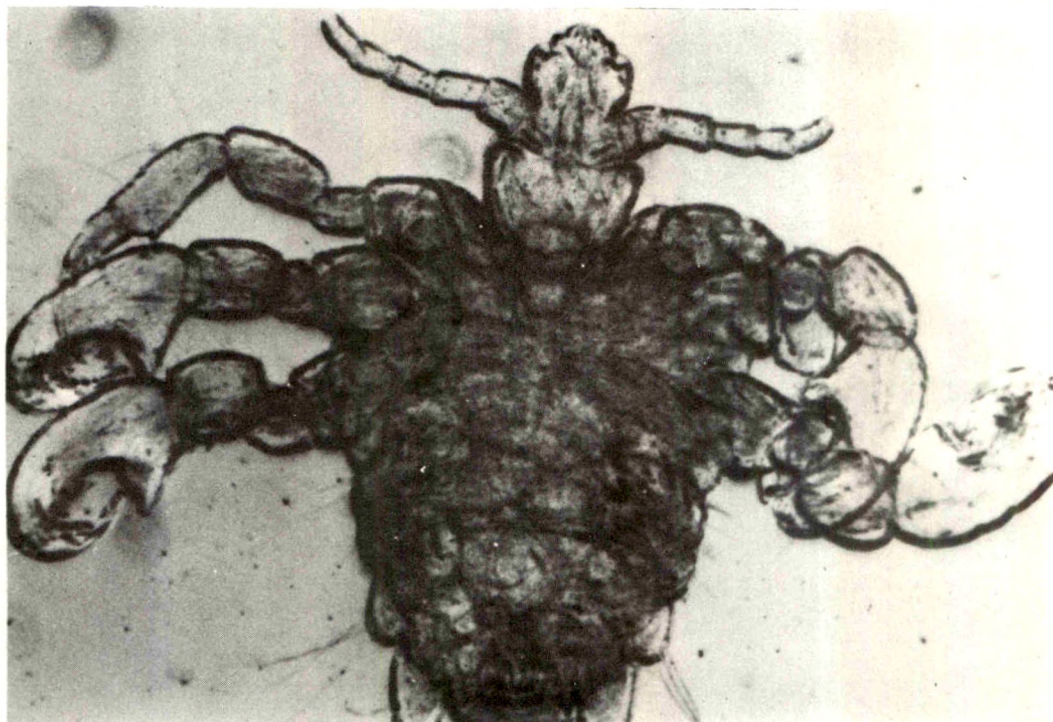
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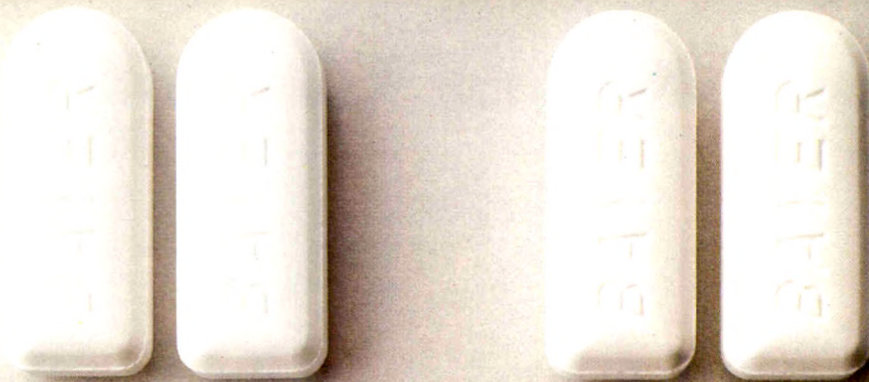
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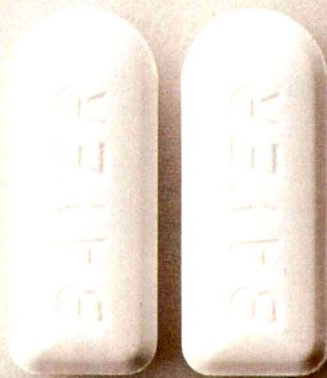
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Evaluation of microhaemagglutination assay to determine treponemal antibodies in CSF

A. BIRRY AND S. KASATIYA

From the Laboratory Services, Quebec Department of Social Affairs, Ste-Anne-de-Bellevue, Canada

SUMMARY The microhaemagglutination assay for *Treponema pallidum* antibodies (MHA:TP-CSF), the Venereal Disease Research Laboratory (VDRL-CSF), and the fluorescent treponemal antibody (FTA-CSF) tests were used to detect treponemal antibodies in 3157 samples of cerebrospinal fluid from patients with syphilis, with neurological, metabolic, or immunological disorders, and with undetermined diagnoses. An agreement of 99.5% between the results of the MHA:TP-CSF and FTA-CSF tests was obtained for all the CSF samples whereas that of only 95.5% was obtained for samples from the known cases of syphilis. The MHA:TP-CSF test is simple, economical, rapid, and specific but not as sensitive as the FTA-CSF test.

Introduction

The *Treponema pallidum* microhaemagglutination assay (TPHA), a relatively simple, highly specific, and sensitive test, was developed to determine the presence of syphilitic antibodies in serum (Rathlev, 1967). Later, many laboratories throughout the world started evaluating this test using a commercial antigen (manufactured by Fujizoki Pharmaceutical Company, Tokyo, Japan). Various authors compared the results of this test with those of the fluorescent treponemal antibody absorption (FTA-ABS) test, which until now was considered to be the confirmatory test, and found an agreement from 85% to 100% for antibodies present in the serum of syphilitic and nonsyphilitic patients (Cox *et al.*, 1971; Garner *et al.*, 1972; Kopecky *et al.*, 1977; Luger and Spendingwimmer, 1973; O'Neill *et al.*, 1973; Sequeira and Eldridge, 1973). Studies indicate that the TPHA test is less sensitive than the FTA-ABS test in detecting cases of primary syphilis (Tomizawa, 1969; Sequeira and Eldridge, 1973; Lesinski *et al.*, 1974). False positivity to the TPHA test in sera of healthy individuals is very rare (Coffey *et al.*, 1972; Garner *et al.*, 1973; O'Neill *et al.*, 1973; Young *et al.*, 1974), but it may frequently be observed in patients with autoimmune diseases (Lesinski *et al.*, 1974).

While much information is available on the evaluation of the TPHA test in serum, little is

described about its application in cerebrospinal fluids (CSF). Jaffe *et al.* (1978) found no reactivity for the TPHA test in CSF from 15 patients with latent syphilis, whereas Monsiorska-Borowska (1977) found it to be the most sensitive of the Venereal Disease Research Laboratory (VDRL), *Treponema pallidum* immobilisation (TPI), and FTA-ABS tests carried out on 110 samples of spinal fluid and 49 sera from patients with suspected or diagnosed late syphilis.

The present study was undertaken to evaluate the reactivity of the microhaemagglutination test in cerebrospinal fluid (MHA:TP-CSF) from non-syphilitic and syphilitic patients at different stages and to compare it with that of the VDRL-CSF and FTA-CSF tests.

Material and methods

During the period of two years, from July 1976 to July 1978, cerebrospinal fluid specimens from 3157 patients were examined in the central laboratory for routine syphilis serology. Of these patients, 356 were known cases of syphilis confirmed by clinical examination, case history, and serology. The remaining 2801 specimens came from patients with undetermined diagnoses and from patients with neurological, metabolic, or immunological disorders. Most of the specimens were collected from the local hospitals and brought to the laboratory within 24 hours of collection. The specimens were kept at -20°C until tested. No information was sought regarding the reactivity of the VDRL, FTA-ABS, and MHA:TP tests in the sera of these cases.

Address for reprints: Dr S. Kasatiya, Ontario Public Health Laboratory, PO Box 6301, 346 Moodie Drive, Ottawa, Ontario K2A 1S8, Canada

Received for publication 3 October 1978

Standard techniques were used for the VDRL-CSF (CDC, 1969) and MHA:TP-CSF (CDC, 1973) tests, whereas the FTA-CSF test was carried out according to the methods described by Duncan *et al.* (1972) and Leclerc *et al.* (1978). During this period all the samples were examined simultaneously by the three tests. The MHA:TP-CSF test was carried out on uninactivated CSF using antigens and serum controls (supplied by Fujizoki Pharmaceutical Company, Tokyo, Japan).

The test was performed and interpreted according to the directions of the manufacturer as recommended for serum; the initial dilution of 1/20 of the CSF sample was brought to a final dilution of 1/80 after addition of the cells. The FTA-CSF reagents (*T. pallidum* antigen, conjugate, and sorbent) were supplied by Beckman Company, Fullerton, California, USA. The FTA-CSF test was carried out routinely on undiluted, unabsorbed CSF. All the CSF samples giving a reactive result in the routine test were retested after dilution to 1/5 in sorbent (supplied by the manufacturer) before being reported as giving a reactive result. Monospecific IgG conjugate was used in a dilution of 1/2300 with unabsorbed CSF and of 1/600 with absorbed CSF to get the same intensity of fluorescence with strongly reactive, weakly reactive, and non-reactive CSF controls selected in the laboratory. A Leitz Orthoplan microscope equipped with an incident light system was used according to the method described by Kasatiya and Birry (1972).

Results

REACTIVITY IN CSF SAMPLES

Known and unknown diseases

Of the 3157 specimens of CSF from patients with (a) neurological, metabolic, or immunological disorders; (b) different stages of syphilis; and (c) undetermined

diagnoses, 3.1%, 6.4%, and 6.9% gave reactive results to the VDRL-CSF, MHA:TP-CSF, and FTA-CSF tests respectively (Table 1). All of the 217 CSF samples giving a reactive result to the FTA-CSF test came from known cases of syphilis. An agreement of 96.3% was obtained between the VDRL-CSF and MHA:TP-CSF or FTA-CSF tests whereas that of 99.5% was found between the MHA:TP-CSF and FTA-CSF tests. False-positive results were not obtained in the VDRL-CSF, MHA:TP-CSF, or FTA-CSF tests. All of the 16 CSF samples giving a reactive result only in the FTA-CSF test came from known cases of syphilis.

Syphilis

The reactivity of the VDRL-CSF, MHA:TP-CSF, and FTA-CSF tests in CSF samples from 356 known cases of syphilis are given in Table 2. The VDRL test gave a reactive result in only 27.5% of cases whereas in the MHA:TP-CSF and FTA-CSF tests 56.5% and 60.9% of samples respectively gave reactive results. CSF samples from 16 patients gave reactive results in only the FTA-CSF test. A poor agreement of 66.6% was obtained in this group between the VDRL-CSF and MHA:TP-CSF or FTA-CSF tests. CSF samples from 139 (39%) patients with syphilis did not show any reactivity in any of the three tests.

Distribution according to sex

Percentage reactivity for the three tests in CSF samples from the 275 men and 81 women with syphilis was almost identical. Of the total 356 CSF samples, 29.4% and 21% in the VDRL-CSF test, 57.4% and 53% in the MHA:TP-CSF test, and 60.7% and 61.7% in the FTA-CSF tests gave reactive results respectively in men and women.

Primary syphilis

All the CSF samples from 32 known cases of primary

Table 1 Results of the VDRL-CSF, MHA:TP-CSF, and FTA-CSF tests performed on CSF samples from 3157 patients

Test results			Total no. of samples		Agreement between tests					
					VDRL: MHA		VDRL: FTA		MHA: FTA	
VDRL	MHA	FTA	No.	%	No.	%	No.	%	No.	%
+	+	+	98	3.1	3054	96.7			3141	99.5
+	+	—	0	0						
+	—	—	0	0						
—	+	+	103	3.3						
—	—	+	16	0.5						
—	+	—	0	0						
+	—	+	0	0			3038	96.3		
—	—	—	2940	93.1						
Total no. reactive (%)			98 (3.1) 201 (6.4) 217 (6.9)							

* Includes two patients with borderline results

+ Positive —negative

Table 2 Results of the VDRL-CSF, MHA:TP-CSF, and FTA-CSF tests performed on 356 CSF samples from known cases of syphilis

Test results			Total no. of samples		Agreement between tests					
					VDRL: MHA		VDRL: FTA		MHA: FTA	
VDRL	MHA	FTA	No.	%	No.	%	No.	%	No.	%
+	+	+	98	27.5						
+	+	—	0	0	253	71.0				
+	—	—	0	0						
—	+	+	103	28.9					340	95.5
—	—	+	16	4.5						
—	+	—	0	0						
+	—	+	0	0						
—	—	—	139	39.0			237	66.6		
Total no. reactive (%)			98(27.5) 201(56.5) 217(60.9)							

*Includes two patients with borderline results

+ Positive —negative

syphilis gave negative results to the VDRL-CSF, MHA:TP-CSF, and FTA-CSF tests.

Secondary syphilis

As many as 50.6% and 64.5% of the CSF samples gave reactive results in the MHA:TP-CSF and FTA-CSF tests respectively, even in cases of secondary syphilis. The VDRL-CSF test gave reactive results in only 20.2% of cases. An agreement between the VDRL-CSF and MHA:TP-CSF or FTA-CSF tests was obtained in 55.7% of cases. A good agreement was found between the MHA:TP-CSF and FTA-CSF tests in this early stage of syphilis. CSF samples from 11 cases of secondary syphilis gave reactive results only in the FTA-CSF test (Table 3).

Tertiary syphilis

All cases of asymptomatic and symptomatic neurosyphilis and cardiovascular syphilis were grouped in this category. It is in this category that most of the CSF samples gave reactive results. Of the

121 CSF samples 91.7% gave reactive results in the FTA-CSF test while 88.4% and 57.9% gave reactive results in the MHA:TP-CSF and VDRL-CSF tests respectively (Table 4). Slightly better agreement was obtained between the VDRL-CSF and MHA:TP-CSF or FTA-CSF tests and between the MHA:TP-CSF and FTA-CSF tests than in cases of secondary syphilis. Of the four patients with reactive results in only the FTA-CSF test, one patient had meningovascular neurosyphilis and three asymptomatic neurosyphilis. The VDRL-CSF test was 2½ times more reactive in tertiary than in secondary syphilis.

Latent syphilis

The reactivity for the VDRL-CSF test dropped markedly to 9.5% in this particular group. The diminution in percentage reactivity was also observed in the MHA:TP-CSF and FTA-CSF tests (Table 5); 53.4% of the CSF samples gave no reactive results in any of these tests. Although agreement between the

Table 3 Results of the VDRL-CSF, MHA:TP-CSF, and FTA-CSF tests performed on CSF samples from 79 cases of known secondary syphilis

Test results			Total no. of samples		Agreement between tests					
					VDRL: MHA		VDRL: FTA		MHA: FTA	
VDRL	MHA	FTA	No.	%	No.	%	No.	%	No.	%
+	+	+	16	20.2						
+	+	—	0	0	55	69.6				
+	—	—	0	0						
—	+	+	24	30.4					68	86.0
—	—	+	11	13.9						
—	+	—	0	0						
+	—	+	0	0						
—	—	—	28	35.4			44	55.7		
Total no. reactive (%)			16 (20.2) 40 (50.6) 51 (64.5)							

+ Positive —negative

Table 4 Results of the VDRL-CSF, MHA:TP-CSF, and FTA-CSF tests performed on CSF samples from 121 known cases of tertiary syphilis

Test results			Total no. of samples		Agreement between tests					
					VDRL: MHA		VDRL: FTA		MHA: FTA	
VDRL	MHA	FTA	No.	%	No.	%	No.	%	No.	%
+	+	+	70	57.9	80	66.1			117	96.7
+	+	—	0	0						
+	—	—	0	0						
—	+	+	37	30.6			80	66.1		
—	—*	+	4†	3.3						
—	+	—	0	0						
+	—	+	0	0						
—	—	—	10	8.3						
Total no. reactive (%)			70 (57.9) 107 (88.4) 111 (91.7)							

* Four cases of +/— reaction reported as a negative result

† One case of meningovascular and three cases of asymptomatic neurosyphilis

+ Positive —negative

Table 5 Results of the VDRL-CSF, MHA:TP-CSF, and FTA-CSF tests performed on CSF samples from 116 known cases of latent syphilis

Test results			Total no. of samples		Agreement between tests					
					VDRL: MHA		VDRL: FTA		MHA: FTA	
VDRL	MHA	FTA	No.	%	No.	%	No.	%	No.	%
+	+	+	11	9.5	74	63.8			115	99.1
+	+	—	0	0						
+	—	—	0	0						
—	+	+	42	36.2			73	62.9		
—	—	+	1	0.9						
—	+	—	0	0						
+	—	+	0	0						
—	—	—	62	53.4						
Total no. reactive (%)										

+ Positive —negative

VDRL-CSF and MHA:TP-CSF tests remained more or less the same as in the previous cases, the agreement between the MHA:TP-CSF and FTA-CSF tests rose to 99.1%.

Congenital syphilis

Of the eight CSF samples from patients with congenital syphilis only one gave a reactive result in all three tests.

Diseases other than syphilis

No reactivity with any of the three tests was found in CSF samples from 499 patients with diseases other than syphilis (Table 6).

Discussion

Most authors consider that the TPHA technique is as specific and sensitive as the FTA-ABS test in detecting treponemal serum antibodies in syphilis,

except in cases of primary syphilis (Tomizawa *et al.*, 1969; Garner *et al.*, 1973; Kiraly and Prerau, 1974) and that the antibody appears later than the one detected by the FTA-ABS test and probably also by the VDRL test (Rudolph, 1976). On the contrary, Jephcott *et al.* (1977) reported a case in which the TPHA test was the first to give a positive result in serum compared with the VDRL and FTA-ABS tests. Similarly, Monsińska-Borowska (1977) found the TPHA test to be more sensitive than the FTA, TPI, and VDRL tests.

It is known that the central nervous system is affected early in the course of early syphilis (O'Neill *et al.*, 1973; Lejman *et al.*, 1976; Kopecky, 1977), but efficient serological tests are not available for detecting treponemal antibodies in CSF in the early stages of syphilis. Previous work done in this laboratory has shown that the FTA-CSF test is capable of detecting treponemal antibodies in 65.9% of cases of secondary syphilis (Leclerc *et al.*, 1978) and this is confirmed by the present study.

Table 6 Results of the VDRL-CSF, MHA:TP-CSF, and FTA-CSF tests performed on CSF samples from 499 patients with diseases other than syphilis

Diseases	Test results			Total no. of samples	Diseases	Test results			Total no. of samples
	VDRL	TPHA	FTA			VDRL	TPHA	FTA	
Anaemia	—	—	—	4	Labyrinthitis	—	—	—	3
Aphasia	—	—	—	4	Lumbar adherence	—	—	—	8
Ataxia	—	—	—	12	Lupus erythematosus	—	—	—	4
Cardiac insufficiency	—	—	—	7	Medullary compression	—	—	—	4
Cerebral atrophy	—	—	—	6	Meningitis	—	—	—	43
Cerebral metastases	—	—	—	1	Migraine	—	—	—	10
Cerebral syndrome	—	—	—	11	Multiple myeloma	—	—	—	2
Cerebral thrombosis	—	—	—	11	Multiple sclerosis	—	—	—	35
Cerebral tumour	—	—	—	4	Myelitis	—	—	—	13
Dementia	—	—	—	16	Neuritis	—	—	—	12
Diabetes	—	—	—	7	Paralysis	—	—	—	17
Discal hernia	—	—	—	60	Paraplegia	—	—	—	5
Encephalomyeloneuropathy	—	—	—	23	Parkinson's diseases	—	—	—	5
Epilepsy	—	—	—	55	Pick's disease	—	—	—	1
Fever	—	—	—	5	Pneumonia	—	—	—	9
Genital herpes	—	—	—	5	Polyarthritis	—	—	—	3
Hemiplegia	—	—	—	6	Polyneuropathy	—	—	—	5
Hepatitis	—	—	—	1	Portal systemic	—	—	—	
Hydrocephaly	—	—	—	17	encephalopathy	—	—	—	8
Hypertension	—	—	—	6	Reticulopathy	—	—	—	1
Hyperthermia	—	—	—	6	Seizures	—	—	—	19
Hypophyseal tumour	—	—	—	3	Toxoplasmosis	—	—	—	4
Hypophyseal disease	—	—	—	5	Tuberculosis	—	—	—	6
Infectious diseases	—	—	—	3	Vasculitis	—	—	—	3
					Viraemia	—	—	—	1

— Negative

The evaluation of the MHA:TP-CSF test—a simpler and less costly technique than the FTA-CSF test—with 2940 non-syphilitic cases indicates that a false-positive result in this test is very rare and that the test is highly specific for treponemal antibodies. Our data supports the observations of Jaffe *et al.* (1978) regarding the high specificity of the TPHA. It further indicates that this test is sensitive in detecting treponemal antibodies in the early stages of syphilis. It was still not possible to detect antibodies in CSF samples from any of the patients with primary syphilis by the two treponemal tests. At later stages of syphilis the test compares well with the FTA-CSF test, thus supporting the observation of Monsiorska-Borowska (1977). Jaffe *et al.* (1978) did not find any reactivity in patients with latent syphilis, probably because of the small number of cases studied.

The MHA:TP-CSF test results were stable and reproducible even after repetition at long intervals of time, provided that the specimen was stored frozen.

Specificity in the FTA-CSF test increases if the test is performed on CSF samples diluted to 1/5 in sorbent but, in some cases, it may lose sensitivity and give false-negative results. CSF samples from 16 known cases of syphilis, of which 11 were from cases of secondary syphilis, four from cases of tertiary syphilis, and one from a case of late latent syphilis, giving non-reactive results in the VDRL-CSF and MHA:TP-CSF tests and reactive results in the FTA-CSF test, became non-reactive in the latter test when

the CSF was retested after absorption. This finding confirms the results of Duncan *et al.* (1972) who found a reduction in the reactivity of the FTA-CSF test after dilution of the CSF in the sorbent. On the other hand, of the same 16 CSF samples when retested undiluted by the MHA:TP-CSF method, 13 (nine from cases of secondary, three of tertiary, and one of latent syphilis) became reactive, thus indicating that the manufacturer's instructions for serum TPHA, where serum is used at a dilution of 1/20, may not apply to the detection of treponemal antibodies in CSF. Diluting the CSF reduces the concentration of immunoglobulins shown by both the low reactivity in the FTA-CSF test and non-reactivity in the MHA:TP-CSF test. Further studies of the MHA:TP-CSF test using undiluted CSF are in progress.

The reactivity in CSF samples from cases of latent syphilis in all the three tests indicates that those cases were syphilitic and not biological false-positive reactors. In the absence of other features they would have been diagnosed as latent cases without the CSF examination. Some may have been previously treated.

Data indicate that the MHA:TP-CSF test is almost identical in specificity but inferior in sensitivity to the FTA-CSF test. The former test, being simple and more economical to perform, can be easily automated for the routine detection of treponemal antibodies in large numbers of CSF samples.

The authors acknowledge the valuable help of Gérard Larose for frequent discussions and of Danielle Wilson for criticisms and presentation of the manuscript and secretarial assistance.

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Attachment of *Neisseria gonorrhoeae* to human sperm

Microscopical study of effect of trypsin and iron

CLARA I. GOMEZ, WAYNE A. STENBACK, ANN N. JAMES,
B. SUE CRISWELL, AND ROBERT P. WILLIAMS

From the Departments of Microbiology and Immunology, Baylor College of Medicine; and the Department of Pathology, Texas Children's Hospital, Houston, Texas, USA

SUMMARY Pilated *Neisseria gonorrhoeae* of colony type 1 (T1) and non-pilated bacteria of colony type 4 (T4) were observed by transmission (TEM) and scanning electron microscopy (SEM). No pili were observed on T4 gonococci, but two types of pili—straight, type a, and bent, type b—were seen on T1 by TEM. When incubated with human sperm and examined by either TEM or SEM, T1 gonococci were seen to attach by individual pili, by several pili wound together as a rope, or by direct contact. Gonococci from T4 colonies attached only by direct contact. Treatment with trypsin (1 mg/ml) damaged or removed pili from gonococci. After incubation with trypsin, attachment of pilated gonococci to sperm was decreased significantly, but such treatment did not affect attachment of non-pilated gonococci. Incubation of gonococci from either colony type in 0.1 mmol/l ferric nitrate, followed by incubation with sperm, significantly increased attachment of only T4 bacteria. No pili were seen on T4 gonococci treated with ferric nitrate; thus, it appears that factors other than pili alone are concerned in attachment of these gonococci to sperm.

Introduction

Occurrence of pili in the genus *Neisseria* was first reported by Wistreich and Baker (1971). *Neisseria* with pili caused haemagglutination, and scanning electron microscopy (SEM) showed that bacterial pili were adhered to the erythrocytes. Novotny *et al.* (1975) distinguished three types of pili on negatively stained *Neisseria gonorrhoeae*. He named these types a, b, and c, according to the occurrence, structure, and serological properties. The most common, type a, showed no tendency to form constant-angle, layered crystal aggregates and could be classified as type II or III according to the nomenclature of Brinton (1965, 1967). Another type composed of shorter units with structures usually bent and segmented was called b, and pili which showed small knobs at the end were designated type c.

Kellogg *et al.* (1962, 1968) described four morphological types of *N. gonorrhoeae*. Colony type 1 (T1) and type 2 (T2) were isolated primarily from exudates of patients and type 3 (T3) and type 4 (T4) developed in laboratory strains. Virulence was associated with T1 and T2, since only these types caused gonorrhoea when inoculated into human volunteers (Kellogg and Thayer, 1969). Swanson *et al.* (1971) later demonstrated the presence of pili on *N. gonorrhoeae* from colony types 1 and 2 that were associated with virulence; pili were not present on the avirulent colony types 3 and 4. Gonococcal pili were purified (Buchanan *et al.*, 1973; Buchanan, 1975) and shown to exist in antigenically (Buchanan, 1975; Robertson *et al.*, 1977) and immunologically (Novotny and Turner, 1975) distinct groups.

The pathophysiology of gonorrhoea has been a puzzle to investigators. Many studies have shown that pili are important in the interaction of gonococci with cells of the potential host. Pili mediate interaction *in vitro* between gonococci and tissue cells (Swanson, 1973), urethral mucosal cells (Ward and Watt, 1972), red blood cells (Punsalang and Sawyer, 1973), leucocytes (Punsalang and Sawyer, 1973;

Address for reprints: Dr A. N. James, Department of Microbiology and Immunology, Baylor College of Medicine, Houston, Texas 77030, USA

Received for publication 14 November 1978

Swanson *et al.*, 1974), human epithelial cells (Punsalang and Sawyer, 1973), and human sperm (James-Holmquest *et al.*, 1974). Attachment of gonococci to human sperm *in vitro* (James-Holmquest *et al.*, 1974) was studied as a model for the host-parasite interaction. Such attachment *in vivo* could partly explain transmission of the bacteria and spread of the disease in the host.

Attachment of gonococci to human sperm is influenced by a variety of physical and chemical factors (James *et al.*, 1976). The enzyme, trypsin, reduced attachment of T1 bacteria to the level of T4, apparently by action on the proteins of pili. Iron salts, in contrast, enhanced attachment. In the present investigation, we examined by electron microscopy the interaction of gonococci with human sperm after treatment of the bacteria with either trypsin or iron salts.

Materials and methods

GROWTH AND STRAINS OF BACTERIA

Colony types 1 and 4 of *N. gonorrhoeae* strain WP were obtained from a clinical isolate stabilised in this laboratory after 30 transfers (James *et al.*, 1973). Cultures were incubated in candle jars for 18-20 hours at 35°C and were maintained by transfer on GC Agar Base Medium (Baltimore Biological Laboratories, Cockeysville, Maryland) plus 1% IsoVitaleX (BBL), a medium we term GCB. Colonies were typed by the method of Kellogg *et al.* (1968) using oblique illumination from beneath the Petri dish. Stocks of *N. gonorrhoeae* were frozen in a solution of four parts of Trypticase Soy Broth (BBL) to one part of glycerol (Mallinckrodt Inc., Paris, Kentucky) and stored at -60°C.

Isolated T1 and T4 colonies of gonococci were picked from the agar surface and suspended in either of two buffered solutions. Alsever's solution (AS) was composed of dextrose, 2.05 g; sodium citrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2 \text{H}_2\text{O}$), 0.80 g; NaCl, 0.42 g; and citric acid ($\text{H}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2 \text{H}_2\text{O}$), 0.55 g in 100 ml of deionised water (Alsever and Ainslie, 1941). Ringer's solution (RS) contained NaCl, 8.6 g; KCl, 0.3 g; and $\text{CaCl}_2 \cdot 2 \text{H}_2\text{O}$, 0.33 g per litre of deionised water (Diem and Lentner, 1970). No significant difference in attachment of gonococci to sperm was found when either of the two buffers was used. AS was used in most of the work because better results were obtained in electron microscopy studies, especially when uranyl acetate (UA) was used as a stain.

For experiments, bacterial suspensions were mixed on a Vortex mixer (Vortex Genie Mixer, Scientific Products, Evanston, Illinois) to obtain an even distribution of cells. The concentration of bacteria per ml was determined in a Petroff-Hausser chamber

(Hausser Scientific, Blue Bell, Pennsylvania); then suspensions were diluted to achieve a final concentration of 2×10^8 organisms per ml.

PREPARATION OF SPERM

Samples of human semen with normal sperm counts were provided by the immunology laboratory of the obstetric and gynaecology department of Baylor College of Medicine, Houston Texas. A pool of seminal fluid was diluted in RS or AS and centrifuged at $1500 \times g$ (Model CL, International Equipment Co., Needham Heights, Maryland) for five minutes. The supernatant fluid was decanted, and more RS or AS was added to the pellet of sperm. This suspension was centrifuged again for five minutes and was finally resuspended in AS or RS. The number of washed sperm per ml was determined with a haemocytometer (Spencer Bright-Line American Optical, Instrument Division, Buffalo, New York) and dilutions were adjusted with the buffer solution to obtain a final concentration of 1×10^8 .

MIXTURE OF BACTERIA AND SPERM

Equal volumes (0.5 ml) of suspensions of *N. gonorrhoeae* T1 or T4 (2×10^8 /ml) and sperm (1×10^8 /ml) were mixed and incubated for 15 minutes at 35°C in a waterbath shaker (Model 676, New Brunswick Scientific Co. Inc., New Brunswick, New Jersey). Samples were then processed for observation by either transmission electron microscopy (TEM), scanning electron microscopy (SEM), or light microscopy (LM).

USE OF PHYSIOLOGICAL AGENTS

The effects of adding trypsin and ferric nitrate to T1 and T4 organisms alone and in combination with sperm were determined. Trypsin from bovine pancreas type III (Sigma Chemical Co., St Louis, Missouri) was prepared as a stock solution of 10 mg/ml by dilution in water and stored at 0°C. A final concentration of 1 mg/ml was used with bacteria.

Ferric nitrate ($\text{Fe}(\text{NO}_3)_3 \cdot 9 \text{H}_2\text{O}$; Fisher Scientific Co., Fairlawn, New Jersey) was prepared by dilution in water to 1 mmol/l, sterilised by filtration (Nalgene filter 0.20 μm , Nalge-Sybron Corp., Rochester, New York) and stored at 4°C in a light-proof container. A final concentration of 1 mmol/l was used in each test.

Both T1 and T4 gonococci were incubated separately for 15 minutes at 35°C with the agent to be tested. After this treatment, the bacteria were washed twice by centrifugation at 19 000 r/min (RC2-B, Sorvall Inc., Norwalk, Connecticut) for 15 minutes each and then suspended to a volume of 1 ml in RS or AS.

PREPARATIONS FOR ELECTRON MICROSCOPY

Scanning electron microscopy

Samples of bacteria, either alone or with sperm suspended in AS or RS, were placed on glass slides and exposed to 1% osmium tetroxide (OsO_4) vapours for 30 minutes. Specimens were dehydrated through 25, 50, 75, 95, and 100% ethanol (v/v) for five minutes each and amyl acetate (100%) for five minutes. The slides were transferred to a critical-point drying apparatus (CPD-1, Denton Vacuum Inc., Cherry Hill, New Jersey), where the amyl acetate was replaced by CO_2 . The critical temperature and pressure changed the liquid to gas in about 30 minutes. This procedure avoided surface tension distortion (Anderson, 1951). The specimens were then coated with gold-palladium and examined with an ETEC scanning electron microscope (ETEC Corp., Hayward, California) with a 20-30° angle of tilt, at an accelerating voltage of 20 kV.

Transmission electron microscopy

Negative staining. Aqueous phosphotungstic acid (PTA) was used in one procedure. A grid coated with carbon-collodion was floated on top of a drop of sample in RS for one minute. The excess fluid was absorbed with blotting paper, and the grid was inverted on a drop of 1% PTA (pH 7.2) for one minute. The excess fluid was blotted up and the grids were allowed to dry in air.

Positive staining. A pseudoreplicative technique (Smith *et al.*, 1961) was used, in which one drop of sample in AS was placed on top of a 2.5-mm thick square of 2% (w/v) Noble agar (Difco Laboratories, Detroit, Michigan) on the end of a glass slide. The preparation was allowed to stand until dry. Samples were exposed to OsO_4 vapours for 15 minutes. The specimen was then covered with 0.75% (w/v) collodion, the excess collodion was drained off, and the preparation was allowed to dry in a vertical position. The preparation was gently immersed in UA (1-2%), whereby the collodion film with embedded organisms floated from the agar block and remained on the stain for two minutes. A carbon-collodion-coated grid was centred over the specimen. The grid was then removed by means of a small peg, and, by one continuous motion, the grid was brought out of the stain with the organisms now on the top. Excess fluid was drained off, and the preparation was allowed to dry in air before being examined in a Siemens 1A electron microscope at an accelerating voltage of 80 kV.

EVALUATION OF ATTACHMENT OF BACTERIA TO SPERM

Percentage of attachment of bacteria to sperm was evaluated by light and transmission electron

microscopy. Statistical analyses showed no significant difference between the two methods.

For LM, clean slides were prepared. Three drops of the mixture of sperm and bacteria after incubation were pipetted on to the top of the slide and allowed to dry in air. The slides were then fixed in 95% (v/v) aqueous ethanol for seven minutes and rinsed with water. Slides were stained with crystal violet, 2 g in 20 ml of 95% ethyl alcohol (Piekarski and Ruska, 1939) for two minutes, rinsed with water, dried in air, and then examined with the oil immersion lens by LM. Several fields were examined until 100 sperm had been counted. The sperm with bacteria attached were enumerated and the percentage of attachment was calculated (James-Holmquest *et al.*, 1974).

For evaluation of attachment by TEM, several grids were stained by the UA method and were observed at a magnification of $\times 8000$. One hundred sperm were examined. Sperm with or without bacteria attached were counted to calculate the percentage of attachment.

DETERMINATION OF PILI DIAMETER

The transmission electron microscope was first calibrated by means of a grating replica with a spacing of 462.9 nm. Micrographs of both the grating replica and pilated bacteria were taken at $\times 20\,000$ magnification. The thickness of pili was obtained by measuring the centre-to-centre width of pili lying side by side. Dimensions were calculated from the grating replica standard.

PERCENTAGE OF GONOCOCCI WITH PILI AND MEAN NUMBER OF PILI/BACTERIUM

T1 bacteria were obtained from pure cultures after incubation at 35°C for 20 hours and were suspended in AS to a concentration of 2×10^8 /ml. Grids were prepared, stained with UA, and examined by TEM. A total of 84 bacteria was examined for the presence or absence of pili. The percentage of bacteria with or without pili was calculated. The number of gonococci with pili divided by the total number examined and multiplied by 100 gave the percentage with pili. The combined total number of pili divided by the number of bacteria from three experiments gave the mean number of pili/bacterium.

Results

Approximately 71.43% of T1 gonococci had pili (Table). The number of pili per bacterium varied considerably from as few as one to more than 40 with an average of 10 per gonococcus. The thickness of pili was 4.5-5.5 nm. Designation of pili was made according to different types observed in TEM. Straight, type a (Fig. 1) pili and bent, type b (Fig. 2)

Table Mean number of gonococci with pili from T1 colonies and mean number of pili/gonococcus as determined by TEM

Experiment no.	Total no. of gonococci	With pili		Without pili		Total no. of pili	Mean no. of pili/gonococcus (range)
		No.	%	No.	%		
1	37	25	67.57	12	32.43	239	9.56 (1-40)
2	14	10	71.43	4	28.57	92	9.20 (1-25)
3	33	25	75.76	8	24.24	272	10.88 (1-24)
Total	84	60	71.43	24	28.57	603	10.05

pili, as described by Novotny (1975), were observed. Spherical structures were seen but not at the end of pili (Fig. 2). Many of these structures appeared as isolated blebs that might be extensions of the bacterial membranes, as suggested by McGee *et al.* (1976).

ATTACHMENT TO SPERM

Piliated *N. gonorrhoeae* mixed with sperm and incubated for 15 minutes at 37°C showed various forms of attachment to sperm. The gonococci attached to sperm tails by several pili twisting together as a rope (Fig. 3a and 3b), by pili in a

spiderweb arrangement around the bacteria (Fig. 4a and 4b), by direct contact alone or along with pili, and by a combination of pilus, ropelike bundles, and direct contact (Fig. 5). In some preparations, the tails of sperm appeared to be wound up by the pilated gonococci (Fig. 6). When non-piliated gonococci and sperm were observed, direct contact was the only type of attachment seen, whereas pilated organisms attached to sperm heads directly or by ropes of pili.

Statistically significant differences in attachment to sperm between gonococci from colony types 1 and 4 were observed (Fig. 7). The mean percentage of sperm with pilated organisms attached was

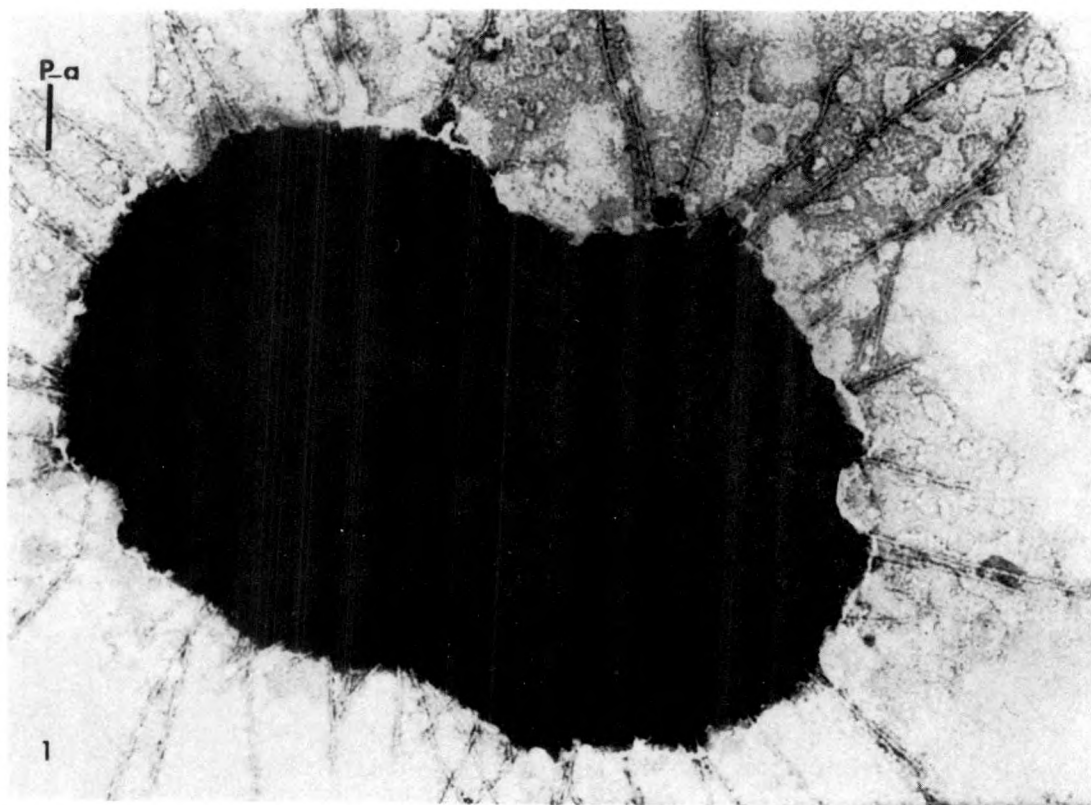


Fig. 1 TEM micrograph of pilated *Neisseria gonorrhoeae* negatively stained by PTA showing pili of type a (P-a). ($\times 80\,000$ magnification).

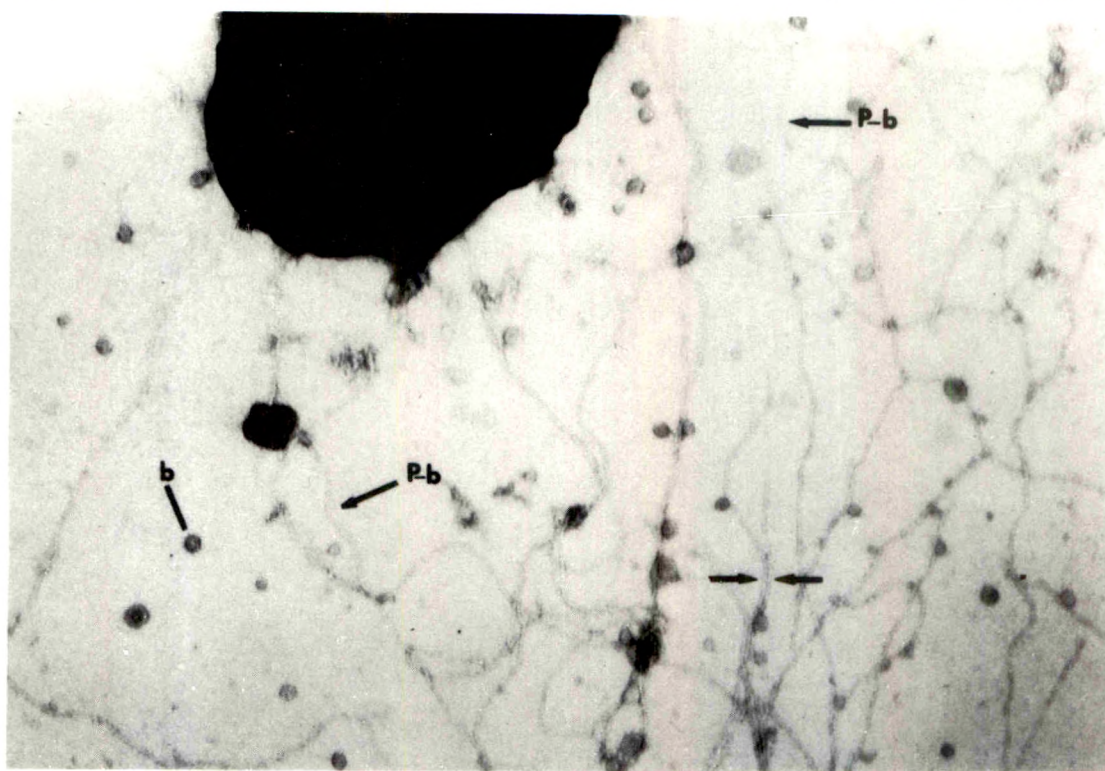
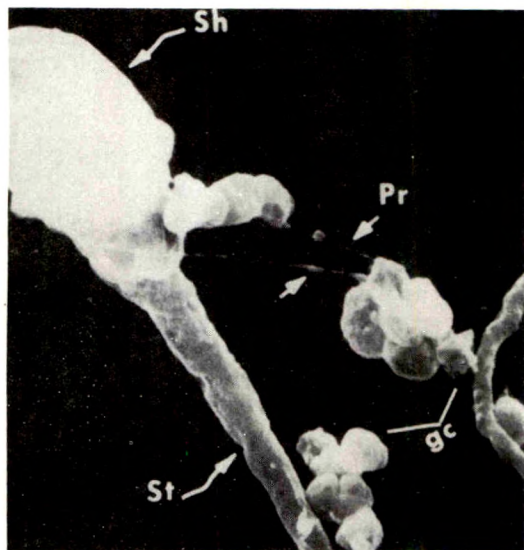
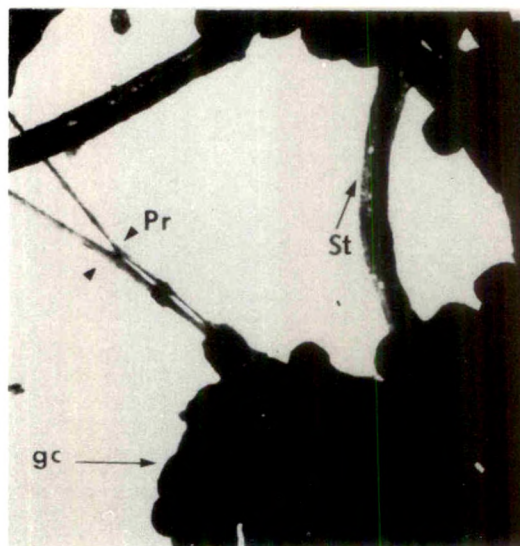


Fig. 2 TEM micrograph of pilated *N. gonorrhoeae* negatively stained with UA. ($\times 31\,000$ magnification). Diameter of pili was obtained by measuring the distance from centre to centre of pili lying side by side (arrows). Pili type b (P-b) and blebs (b) are visible in this micrograph.



(a)



(b)

Fig. 3 Micrographs of pilated *N. gonorrhoeae* attached to sperm by direct contact and by ropes of pili: (a) SEM micrograph ($\times 9000$ magnification); (b) TEM micrograph ($\times 13\,500$ magnification). Arrows indicate sperm head (Sh), sperm tail (St), gonococci (gc), and ropes of pili (Pr).

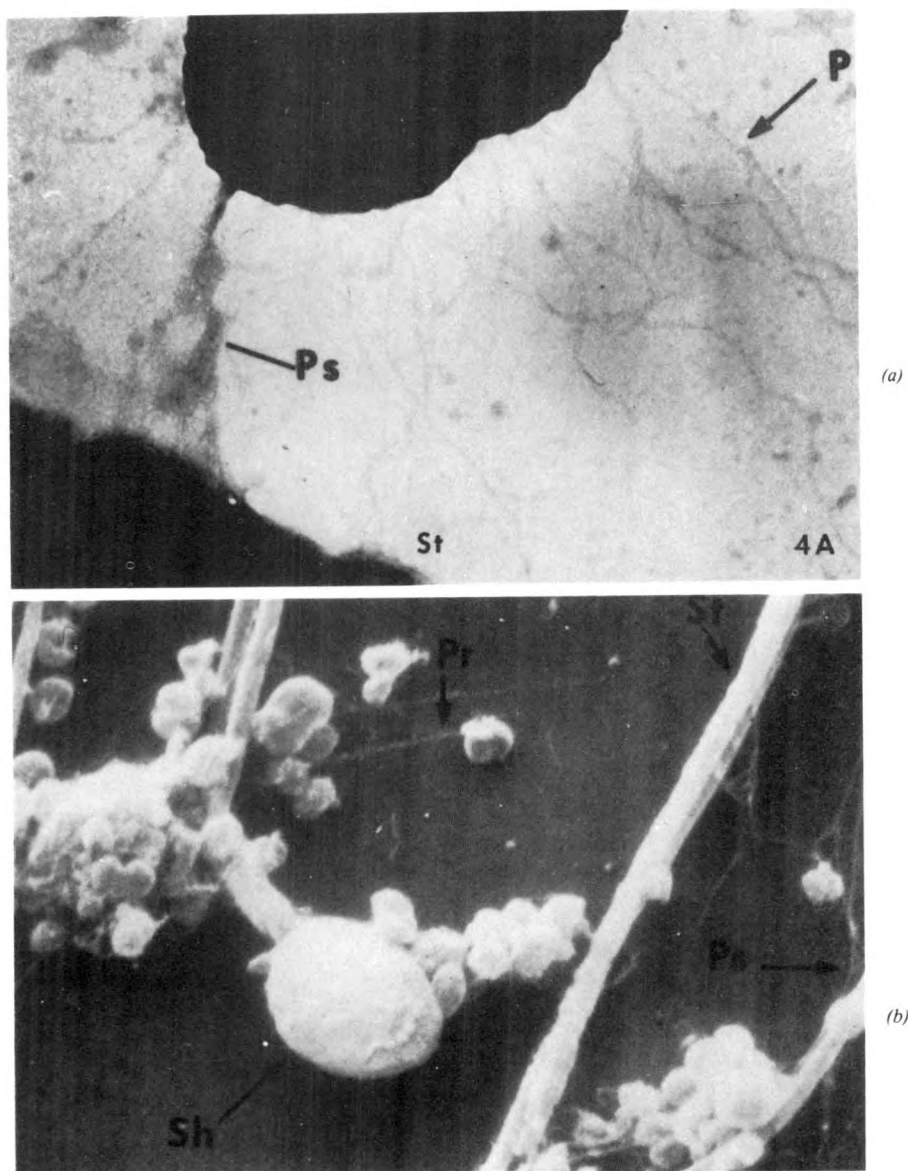


Fig. 4 Micrographs of pilated (*P*) *N. gonorrhoeae*: (a) TEM micrograph showing spiderweb (*Ps*) arrangement of attachment to sperm tail (*St*) ($\times 52\,000$ magnification); (b) SEM micrograph showing spiderweb arrangement (*Ps*) and ropes of pili (*Pr*) and direct attachment to sperm head (*Sh*) and to sperm tail (*St*) ($\times 7000$ magnification).

$61.8 \pm 2.4\%$ by LM and $62.6 \pm 2.06\%$ by TEM. The mean percentage of sperm with non-pilated gonococci attached was $41.3 \pm 3.2\%$ and $41.5 \pm 2.1\%$ by LM and TEM respectively. Results analysed by Student's *t* test showed a significant difference ($P = < 0.0005$ for both LM and TEM) between the percentage of attachment. The two methods of microscopy were comparable because no

significant variations were detectable in the results obtained by either LM or TEM.

EFFECT OF TRYPSIN

Pilated gonococci treated with trypsin showed both degradation and loss of pili (Fig. 8). Adherence to sperm was mostly due to direct contact, and mediation by pili was rarely observed. After

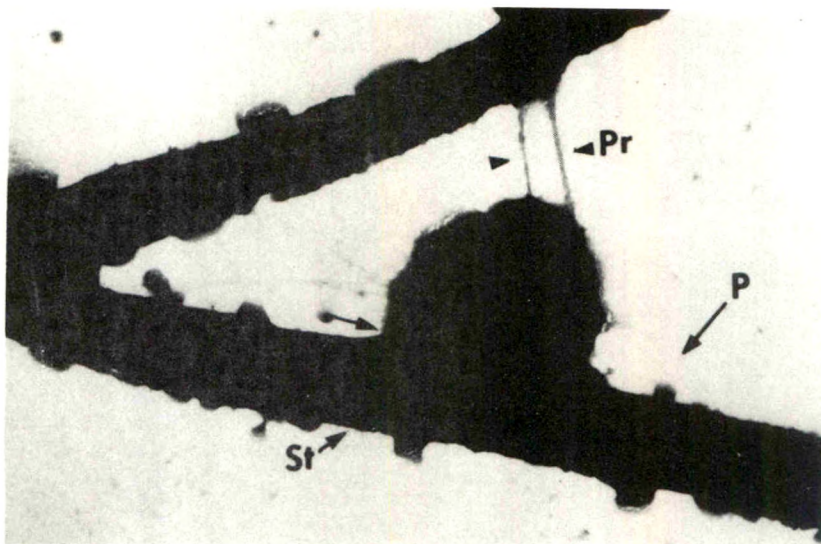


Fig. 5 TEM micrograph of pilated *N. gonorrhoeae* attached to sperm tail (St) by direct contact (arrow), individual pilus (P), and ropes of pili (Pr). ($\times 31\,500$ magnification).

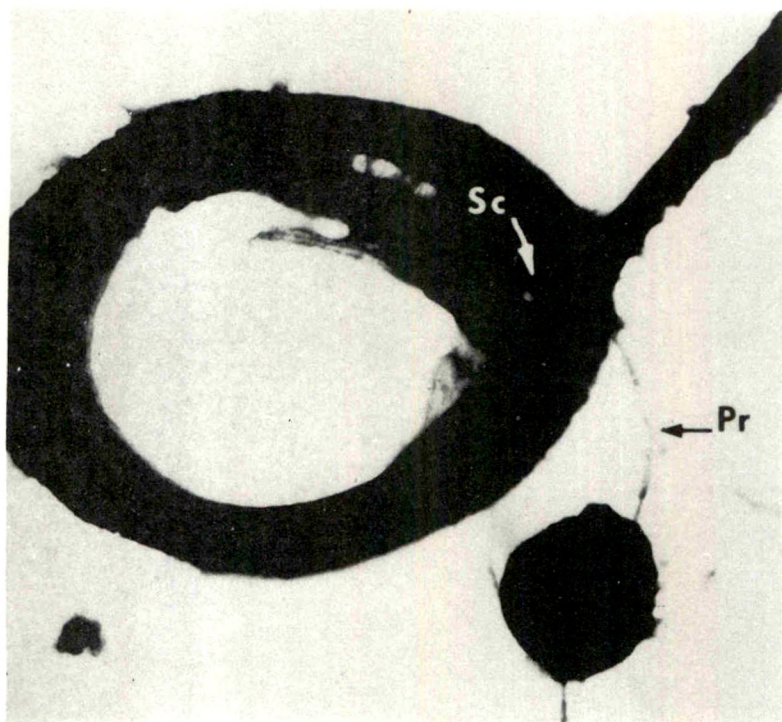


Fig. 6 TEM micrograph of pilated *N. gonorrhoeae* attached to a coiled sperm tail (Sc) by ropes of pili (Pr). ($\times 26\,400$ magnification).

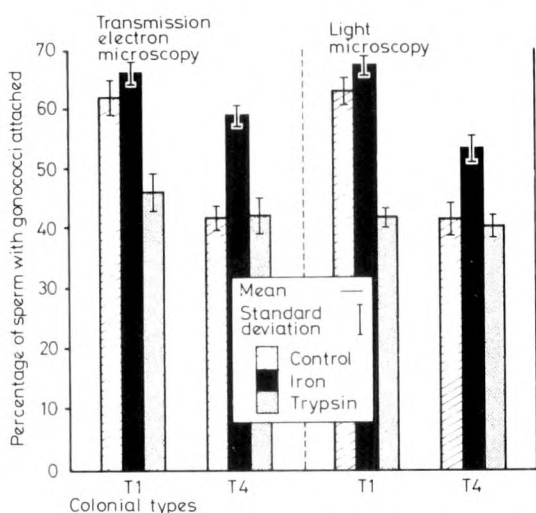


Fig. 7 Comparison by TEM and LM of the percentage of sperm with attached gonococci from T1 or T4 colony types. Attachment by bacteria treated with iron, or trypsin, before incubation with sperm is contrasted with attachment by untreated gonococci (control).

treatment with trypsin and incubation with sperm, pilated organisms showed a significant ($P = < 0.005$) decrease in attachment of 18%, but non-piliated bacteria after the same treatment with trypsin showed a decrease of only 0.6% in attachment ($P = < 0.40$).

EFFECT OF IRON

Iron treatment of T1 gonococci enhanced attachment to sperm by only 4% ($P = < 0.20$). No increase in piliation was observed. Pili of type b, as well as of type a, were still observed (Fig. 9). In contrast, T4 gonococci pretreated with iron salts attached to 15% more sperm than did the control, a significant difference ($P = < 0.0005$). The increase in attachment, however, was not mediated by pili because no pili were visible when specimens of sperm and T4 gonococci treated with iron were observed (Fig. 10).

Discussion

The interaction of gonococci with sperm was examined by three types of microscopy. Each type gave a different dimension to studies of attachment. Specimens dried on glass-slide fragments by the

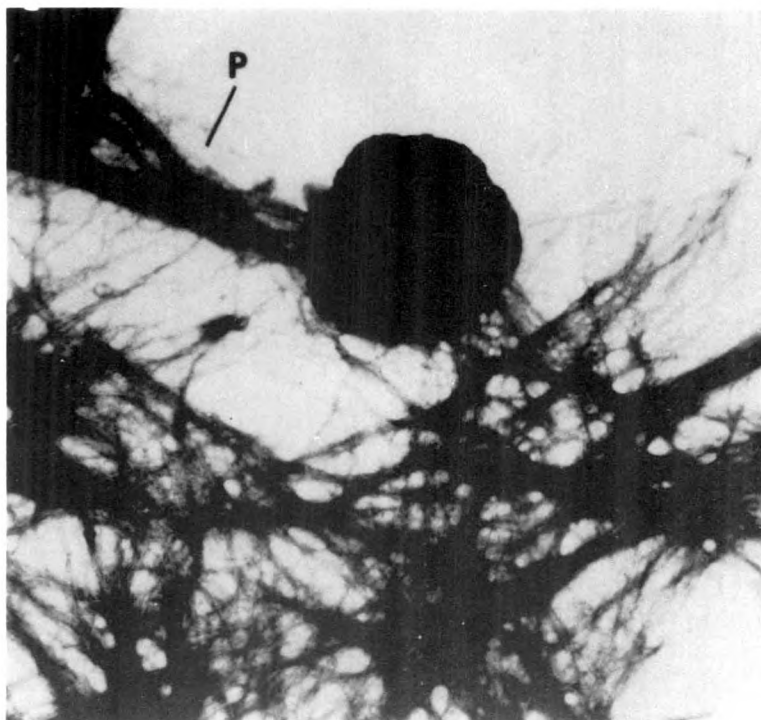


Fig. 8 TEM micrograph of pilated *N. gonorrhoeae* after treatment with trypsin showing partial depilation. The background shows large numbers of pili (P) separated from cells. ($\times 28\,500$ magnification).

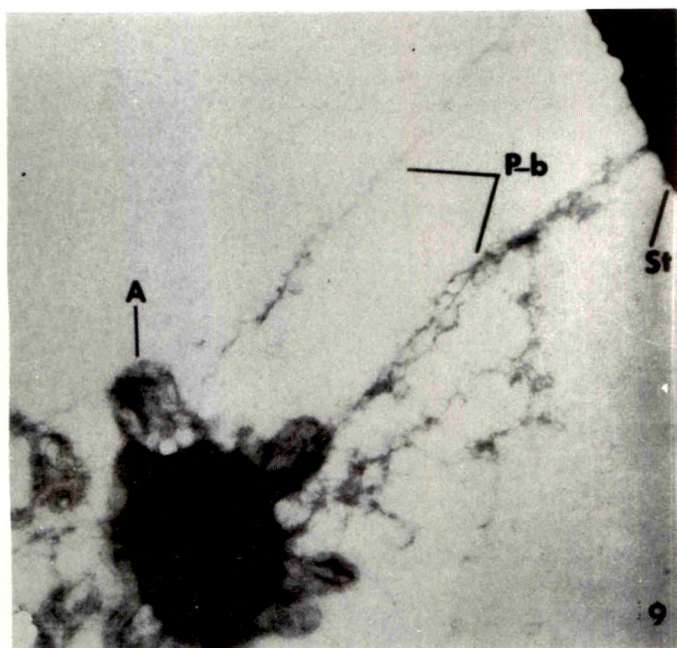


Fig. 9 TEM micrograph of pilated (type b, P-b) *N. gonorrhoeae* treated with iron, incubated with sperm (St), and positively stained with UA. ($\times 36\,600$ magnification).

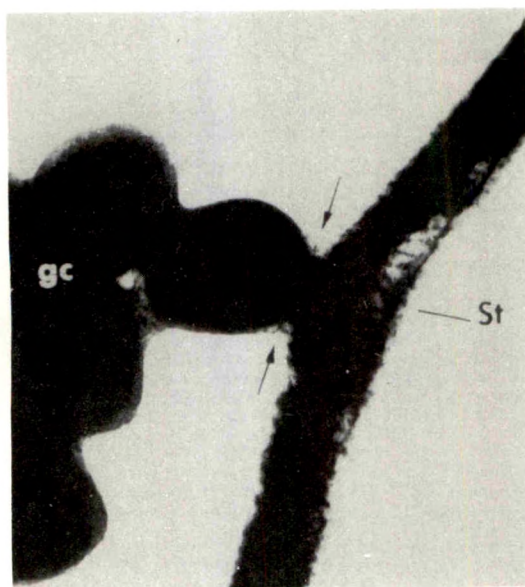


Fig. 10 TEM micrograph of non-piliated *N. gonorrhoeae* treated with iron, incubated with sperm (St), and then negatively stained with UA. Only direct contact (arrows) was observed. ($\times 34\,300$ magnification).

critical-point drying procedure gave the best results for SEM. This type of microscopy is important for observations of the tridimensional structure of both prokaryotic and eukaryotic cells. TEM was useful in

documenting adherence of pilated and non-piliated gonococci to sperm. Light microscopy confirmed the results of James-Holmquest *et al.* (1974) and James *et al.* (1976).

TEM was used in most of the work because the resolving power is greater (0.4–0.8 nm) than SEM (10.0–12.0 nm). The difference in resolution allowed us to observe the ultrastructure of pili and the interaction of gonococci, with or without pili, with sperm. For TEM, several techniques were investigated. The best results were obtained with specimens in Alsever's solution, fixed with osmium vapours, and either negatively or positively stained with uranyl acetate. The application of the pseudoreplicative technique provided a more consistent preparation and minimised interference from the background. Breakage of the coated grid around the bacterial periphery was avoided in most grids by this method. In addition, positively stained pili were well defined. The measurements of pilar diameter in both positively and negatively stained preparations closely agreed.

The diameter of pili varied little from 4.5–5.5 nm. The results agreed with the observations of Jephcott *et al.* (1971), Novotny *et al.* (1975), and McGee *et al.* (1976). Results obtained by Swanson *et al.* (1971) (8.0–10.0 μm) were not comparable, possibly owing to different methods of calibration.

Both straight pili of type a and bent pili of type b were seen (Novotny *et al.*, 1975). These types were observed in different experiments and by the

different techniques (Figs 1, 2, and 6). In contrast to the findings of Novotny *et al.* (1975), type a pili did not appear to be part of the cell wall.

Pili of type c that have a knob at the end (Novotny *et al.*, 1975) were not included in our classification because, although some of our preparations showed similar spherical structures, these rarely occurred at the end of a pilus. Isolated blebs and possible membranous extensions of different sizes and shapes were seen in many micrographs (Figs 2, 4a, and 10). Other investigators have described similar structures Grimble and Armitage, 1974; DeVoe and Gilchrist, 1975; McGee *et al.*, 1976), and lipopolysaccharides were isolated from comparable blebs (Novotny *et al.*, 1975). These blebs and membrane extensions were present on the external surface of the cell wall and occasionally wrapped around the cell (Fig. 10).

Each gonococcus from colony type 1 that possessed pili had an average of 10. We do not know why some lacked pili, although treatment of the specimen for electron microscopy could have removed appendages. The number of pilated gonococci (71%) was greater than the number without.

Removal of pili from gonococci by trypsin reduced attachment to sperm to the level expected for non-piliated bacteria (Punsalang and Sawyer, 1973; James *et al.*, 1976). Ninety per cent of T1 bacteria treated with trypsin attached directly to sperm rather than through the usual mediation by pili. These findings supported the hypothesis that pili play a part in the adherence of gonococci to eukaryotic cells (Swanson, 1973). The important role of pili in attachment is emphasised by the work of James-Holmquest *et al.* (1974) in contrast to the conclusion of Novotny *et al.* (1977).

Attachment of gonococci to sperm appears to involve other factors besides pili. Treatment of avirulent, T4 gonococci with iron salts significantly increased attachment to sperm. In contrast, similar treatment of T1 bacteria had little effect on attachment. We speculate that, if iron is mostly concentrated in the cell membrane (Bartsch, 1968; Lankford, 1973), the polarity of the membrane surface may be changed thereby enhancing adherence (Heckels *et al.*, 1976). Also, changes in the configuration of the cell membrane might make receptor sites more available for adherence by non-piliated gonococci. Of interest for these speculations is the recent report that iron salts increased the killing power of avirulent T3 and T4 gonococci after intravenous inoculation into chicken embryos but had little or no effect on the virulence of T1 and T2 gonococci (Payne and Finkelstein, 1975).

The attachment of pilated gonococci to sperm *in vivo* could affect the fertility of the host as well as

provide bacteria with transportation through the female urogenital tract (Howard, 1971; James-Holmquest *et al.*, 1974). Isolation of T-mycoplasma in men with infertile marriages was correlated with a decrease in sperm motility (Fowlkes *et al.*, 1975b). Our observations of coiled sperm tails that appeared to be wound up by pilated gonococci (Fig. 6) were reminiscent of the reported association of T-mycoplasma with coiled sperm tails (Fowlkes *et al.*, 1975a). A high incidence of gonorrhoea has been associated with low fertility (Arya *et al.*, 1973). Thus, one effect of gonococcal disease could be direct interference with sperm motility by entanglement of sperm tails by the bacteria.

This work was supported in part by Public Health Service Grant AI 12618 from the National Institute of Allergy and Infectious Diseases and by NASA Contract NAS 9 13139. We acknowledge the excellent technical assistance of Joe Dardano in preparation of scanning electron micrographs.

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Studies of ciliated epithelia of the human genital tract

3: Mucociliary wave activity in organ cultures of human Fallopian tubes challenged with *Neisseria gonorrhoeae* and gonococcal endotoxin

P.-A. MÅRDH,* B. BALDETORP,† C. H. HÅKANSSON,† H. FRITZ,‡
AND L. WESTRÖM§

From the *Institute of Medical Microbiology, University of Lund, Department of †Oncology and §Obstetrics and Gynecology, University Hospital, Lund, and ‡Department of Bacteriology, Halmstad, Community Hospital, Halmstad, Sweden

SUMMARY Quantitative determinations of the mucociliary activity of human Fallopian tube epithelium maintained as organ cultures were performed using a light beam reflex method. In non-infected organ cultures the mucociliary wave (MCW) frequency slowly decreased during the first 54 hours of culture maintenance. In organ cultures experimentally infected with fresh isolates of *Neisseria gonorrhoeae* producing T1/T2 colonies the MCW frequency either decreased to subnormal values or completely ceased whereas in organ cultures infected with a laboratory-adapted gonococcal strain the MCW frequencies remained within normal range. In organ cultures exposed to gonococcal endotoxin prepared from the laboratory-adapted strain, as well as in cultures in which cell-free filtrates of medium from organ cultures infected with *N. gonorrhoeae* (producing T1/T2 colonies) were added to the culture medium, the ciliary activity decreased and subsequently ceased. The same phenomenon occurred when organ cultures were challenged with *Escherichia coli* endotoxin. The ciliostatic effect appeared before any morphological changes in the surface epithelium, including the cilia, were demonstrable by scanning electron microscopy.

Introduction

Infection of the Fallopian tubes occurs in 10-15% of women with cervical gonorrhoea (Falk, 1965; Rees and Annels, 1969). The frequency with which *Neisseria gonorrhoeae* causes the salpingitis in these patients, however, may be considerably lower (Mårdh *et al.*, 1978a). It is not known what factors promote dissemination of gonococci from the cervix to the tubes, but it is generally accepted that the route of spread is canalicular—that is, *Neisseria gonorrhoeae* infects the tubes from the lumen. In the naturally occurring disease, there is little opportunity for obtaining tubal specimens for studies of the

effects of gonococcal infections on the epithelial lining of the tubes, particularly in mild cases and in the early acute stage of the disease.

Organ cultures of human Fallopian tubes have been used for studies of experimental gonococcal infections (Carney and Taylor-Robinson, 1973; Taylor-Robinson *et al.*, 1974; Ward *et al.*, 1974; Ward and Watt, 1975; Mårdh *et al.*, 1976; McGee *et al.*, 1976, 1978).

Apart from morphological studies, organ cultures of Fallopian tubes provide a means for studying tubal ciliary activity (McGee *et al.*, 1976; Weström *et al.*, 1977). In experimental gonococcal infection of such specimens, determination of this activity has been made with the aid of light microscopy (Taylor-Robinson *et al.*, 1974; McGee *et al.*, 1976). Great precision in measuring ciliary function may be achieved by registering the light reflections from mucociliary waves (MCW) caused by beating cilia (Toremalm *et al.*, 1974).

Address for reprints: P.-A. Mårdh, Institute of Medical Microbiology, University of Lund, Sölvegatan 23, S-223 62 Lund, Sweden

Received for publication 15 August 1978

The aims of the present study were: (a) to study the mucociliary activity, as shown by the MCW pattern, in organ cultures of human Fallopian tube epithelium with the aid of the light beam reflex method; (b) to study the effect of experimental infections with *N. gonorrhoeae* and of gonococcal and *Escherichia coli* endotoxins on the ciliary activity of these specimens; and (c) to study the tubular epithelium maintained as organ culture and the interrelationship between the epithelium and gonococci by means of scanning electron microscopy (SEM).

Material and methods

TUBAL SPECIMENS

Fallopian tubes were obtained from women who had undergone hysterectomy because of non-malignant disease or who were sterilised for non-medical reasons. All women were aged less than 45 years and were still menstruating. None of the women had received hormonal or radiological treatment. None had gonorrhoea. The operations were performed on different days of the menstrual cycles.

ORGAN CULTURE TECHNIQUE

The organ culture technique used was that already described in detail (Mårdh *et al.*, 1976). In the present series of experiments, four specimens were prepared from the ampullary portion of each tube. Two specimens served as controls and two were challenged. The medium used to transport the Fallopian tubes and to maintain them as organ cultures consisted of Earle's balanced salt solution supplemented with amino acids, glutamine, sodium bicarbonate, and vitamins (Flow Laboratories Ltd, Irvine, Scotland). Trimethoprim and polymyxin B were added to the organ culture medium in the same concentrations as used in the gonococcal culture medium. These antimicrobials were present throughout the experimental period.

GONOCOCCI

Some of the strains of *N. gonorrhoeae* used to infect the organ cultures were clinical isolates harvested from the primary isolation plate containing a gonococcal medium which included 25 IU polymyxin B and 3 mg trimethoprim per ml (Mårdh *et al.*, 1978b). The cultures were incubated overnight at 37°C in an atmosphere of 7% CO₂ and of constant humidity. After overnight incubation a few gonococcal colonies were selected and scraped off the agar surface and suspended in the organ culture medium to a density of approximately 5×10^4 organisms/ml. This suspension was used to infect the organ cultures.

In other experiments, two strains (5 and 7) obtained from the Neisseriae Department, Statens Seruminstitut, Copenhagen, Denmark, were used. These strains had been passaged on solid gonococcal culture medium daily for more than 10 years. During the last few years, the strains were cultured on the medium already mentioned (Mårdh *et al.*, 1978b).

The type of the gonococcal colonies (Kellogg *et al.*, 1963) used to infect the organ cultures was determined by examining the solid culture medium with the aid of a stereomicroscope using oblique illumination. Strains 5 and 7 produced T4 colonies. The fresh isolates of gonococci used produced T1 or T2 colonies.

Specimens of medium were collected daily from the organ cultures during the maintenance period. In these, both the number of colony-forming units (cfu) per ml and the colonial type of the gonococci were determined. The tissue culture medium was replaced by fresh (antibiotic-containing) medium after 48 hours of maintenance of the organ cultures.

All organ cultures were checked daily for secondary infection by aerobic and anaerobic culture of the medium on blood agar plates.

In some experiments, medium from organ cultures experimentally infected with freshly isolated gonococci and from non-infected control cultures were collected after 30 hours' incubation of the organ cultures. The specimens were centrifuged and filtered through a 0.45 µm millipore filter (Millipore, Molsheim, France) and kept at -20°C until used to challenge Fallopian tube organ cultures using a concentration of 1/10 (v/v) in fresh medium.

PREPARATION OF ENDOTOXIN

Endotoxin was prepared from strain 5 of *N. gonorrhoeae* by the method described by Maeland (1968). The gonococci were grown for 24 hours on a solid medium (Mårdh *et al.*, 1978b); they were then harvested by sweeping the agar surface with a glass rod which was subsequently shaken in saline. The suspension was centrifuged ($1000 \times g$) for 20 minutes. After 1.15 g (determined as wet weight) of the bacteria had been washed twice and suspended in 4 ml of saline and 8 ml of diethyl-ether the suspensions were kept in sealed tubes at 4°C for 48 hours and occasionally shaken. After centrifugation at $4000 \times g$ for 30 minutes the water phase was collected and 2 volumes of cold acetone were added. The deposit was collected by centrifugation as described and dialysed, using a dialysis bag (pore diameter 24×10^{-1} nm, for five days in distilled water which was changed every second day. The yield was 1 ml per 1.15 g of bacteria. The preparation was diluted to 1/5 in saline and added to the organ culture medium at a final dilution of 1/1000.

E. coli (0111:B4) endotoxin prepared according to Westphal and co-workers (1952) was purchased from Difco (Difco 3122). The endotoxin was dissolved in distilled water to concentrations of 10 and 100 µg/ml before it was added to fresh organ culture medium to final concentrations of 0.2 and 2.0 µg/ml.

DETERMINATION OF CILIARY ACTIVITY

The pattern and the frequency of the mucociliary waves caused by the beating cilia were registered by a light beam reflex method (Håkansson and Toremalm, 1965). The specimens were placed in a chamber at a steady temperature of 37°C and a relative humidity of 90%. A fibre optic system (Schott-Mainz, model KL 150, Zeiss, West Germany) was used as light source. The light reflections from suitable spots on the tubal epithelial surface were focused by means of an operating microscope (model OPMI 1, Zeiss), which had a diaphragm with a central aperture of 0.1 mm in diameter mounted on the ocular. A photomultiplier (EMI 9524 B, Middlesex, England) was fitted on the top of the ocular diaphragm. The photomultiplier was fed with 1000 V d.c. from a power supply (Oltronix A 2.5L-10 HR, Stockholm). The impulses were then filtered with a Krohn-Hite 3550 filter (Cambridge, Massachusetts, USA) in band pass mode 3-30 Hz. An Elema-Schönander Mingograph 34 (Stockholm) was used for recording the light reflection variations.

The MCW activity of both non-infected and experimentally challenged tubal specimens was registered after the organ cultures had been maintained for 6, 18, 30, 42, and, in some instances, 54 hours. For each specimen studied the numerical value given for the MCW frequency was the mean of six registrations from different spots on the tubal epithelium.

SPECIMEN PREPARATION FOR SCANNING ELECTRON MICROSCOPY

Organ culture specimens were fixed, dried, and subjected to critical-point drying (Mårdh *et al.*, 1976). After drying, the specimens were mounted on specimen stubs, covered with gold-palladium in a vacuum evaporator, and examined in a Cambridge Stereoscan Mark II A scanning electron microscope.

Results

MCW FREQUENCIES IN ORGAN CULTURES

Non-infected

The MCW frequencies of Fallopian tube epithelium in 54 non-infected organ cultures from 28 tubes of 14 patients were determined after 6, 18, 30, 42, and 54 hours of culture maintenance. The mean values and standard deviations obtained are shown in Figure 1.

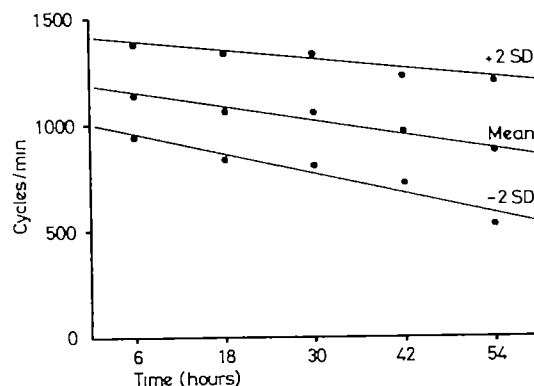


Fig. 1 Mean value (mean) and standard deviation (SD) of MCW frequencies in 54 non-infected Fallopian tube organ cultures from 28 Fallopian tubes maintained for 54 hours. At each registration, the MCW frequency on six different areas of the specimen was registered.

These values were used for reference when studying the experimentally infected organ cultures. In the non-infected reference cultures, the MCW frequencies decreased from a mean of 1161 cycles per minute (c/min) to 894 c/min between six and 54 hours of culture maintenance. In no experiment was a frequency <600 c/min observed. When the frequency had decreased to about 600 c/min the ciliary activity in these non-infected specimens suddenly ceased.

In analyses of the MCW frequencies of the non-infected and experimentally infected organ cultures of epithelium from the same Fallopian tube, only experiments in which the control organ cultures had frequencies within the reference range are reported.

Experimentally infected with N. gonorrhoeae

In four out of eight organ cultures experimentally infected with freshly isolated gonococci (producing T1/T2 colonies), all ciliary activity ceased within six hours, in a further two within 30 hours, and in the remaining two before 42 hours (Table). MCW frequencies within the normal range were not seen after 18 hours.

In all six organ cultures experimentally infected with the gonococcal strain 5, ciliary activity was detectable after 54 hours of culture maintenance (Table). In 26 out of 30 registrations on infected tubal specimens, the MCW frequencies were within the reference range although they were lower than in the control cultures (Figure 2). The differences between the mean values of control and infected organ cultures increased with time (Figure 2).

Challenged with cell-free filtrates

When Fallopian tube organ cultures were maintained

Table Mucociliary wave activity in Fallopian tube organ cultures

Organ culture challenged with	MCW frequency*	Hours of organ culture maintenance				
		6	18	30	42	54
Freshly isolated gonococci (n = 8)	Within NR†	4	1	0	0	
	Below NR	0	3	2	0	
	None	4	4	6	8	
Laboratory-adapted gonococci (n = 6)	Within NR†	6	6	6	4	4
	Below NR	0	0	0	2	2
	None	0	0	0	0	0
Cell-free filtrates of other cultures infected with <i>N. gonorrhoeae</i> (n = 10)	Within NR	1	0	0	0	
	Below NR	5	6	4	1	
	None	4	4	6	9	
Gonococcal endotoxin (n = 14)	Within NR	6	4	0	0	0
	Below NR	5	5	8	7	4
	None	3	5	6	7	10
<i>E. coli</i> endotoxin (µg/ml) (n = 8):						
	0.2					
	2.0					
	0.2					
	2.0					
	0.2					
	2.0					

* Mean of six determinations on each specimen at the four (or five) indicated readings

† Only experiments in which the control organ cultures had frequencies within the reference range are reported

NR = normal range; n = no. of experiments

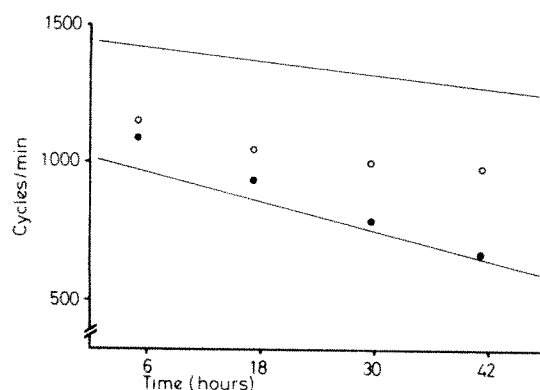


Fig. 2 Mean differences between the MCW frequencies of non-infected Fallopian tube organ cultures (○) and simultaneously performed cultures experimentally infected with a laboratory-adapted strain (strain 5) of *Neisseria gonorrhoeae* (●). (The straight lines indicate mean $\pm$ 2 SD in non-infected cultures.)

in medium containing cell-free filtrates from other cultures infected with freshly isolated gonococci producing T1/T2 colonies the ciliary activity in nine out of 10 organ cultures ceased within 42 hours (Table). Thus the MCW frequencies in eight out of 17 recordings with detectable activity were below 600 c/min—that is, 300, 365, 380, 415, 420, 425, 516, and 581 c/min.

In those organ cultures to which cell-free filtrates from non-infected cultures had been added, the

MCW frequencies remained within normal range throughout the period of culture maintenance.

Challenged with gonococcal endotoxin

In none of the 14 tubal organ cultures challenged with gonococcal endotoxin did the MCW frequency remain within the reference range for more than 18 hours (Table). In these specimens the decrease in ciliary activity was gradual. In 29 out of 39 recordings with detectable activity, the MCW frequencies were below the lower limit for the reference range. The subnormal MCW activity ranged between the lower limit for the reference curve and 403 c/min. After 54 hours of culture maintenance, no ciliary activity could be detected in 10 of the 14 organ cultures whereas in all non-infected control cultures the MCW activity was still within the reference range.

Challenged with *E. coli* endotoxin

In all eight organ cultures challenged with *E. coli* endotoxin at a concentration of 0.2 µg/ml medium, the ciliary activity ceased within 42 hours (Table). Similar results were obtained when a concentration of 1.0 µg/ml of the same endotoxin was used. In all but four of the 64 recordings the ciliary activity was either within normal range or entirely absent (Table). The lag before ciliary activity stopped was the same in the experiments with the two different concentrations of endotoxin used.

SCANNING ELECTRON MICROSCOPY

In non-infected Fallopian tube organ cultures, scanning electron microscopy showed generally normal surface structures throughout the culture maintenance period—that is, 54 hours.

In the organ cultures infected with *N. gonorrhoeae*, the mucosal surface structures did not show any changes as regards the non-infected cultures during at least 48 hours of incubation.

No morphological differences between the cilia of infected and non-infected organ cultures could be demonstrated. Scanning electron micrographs showed an unchanged morphology of the cilia throughout the maintenance period of the organ cultures (Fig. 3).

During the first 48 hours of incubation of the organ cultures, the gonococci generally formed micro-colonies on the surface of the secretory cells but never of ciliated cells (Fig. 3). After 48 hours the number of gonococci on the mucosal surface decreased.

When the organ cultures were infected with gonococci forming T1/T2 colonies their diplococcal structure was clearly visible. The gonococci in the micro-colonies were interspersed by fine threads (Fig.

4a). The surface of the gonococci was ragged and, on some, small buds or protrusions could be seen (Fig. 4b).

When gonococci producing T3/T4 colonies were used to infect the organ cultures micro-colonies were also seen. Threads or protrusions did not occur, and the surfaces of the bacteria seemed 'naked' compared with those of gonococci producing T1/T2 colonies (Fig. 5).

RECOVERY OF GONOCOCCI FROM INFECTED ORGAN CULTURES

The numbers of cfu of gonococci which were re-isolated from the media of the organ cultures infected with gonococci producing T1/T2 colonies are given in Figure 6. In 11 out of 17 samples collected during the first 30 hours of incubation, the number of cfu per ml medium was 1-3 log higher than the infective dose, that is, approximately 5×10^4 cfu per ml. At 48 hours the mean number of cfu per ml medium was the same as the infective dose. At 72 hours—that is, 24 hours after changing the tissue culture medium—the samples contained 10^2 - 10^5 cfu per ml (Fig. 6).



Fig. 3. Scanning electron micrograph of micro-colony of gonococci (producing T4 colonies) on the surface of secretory cells of Fallopian tube epithelium maintained as organ culture for 48 hours. (Experimental infection.) ($\times 6000$).

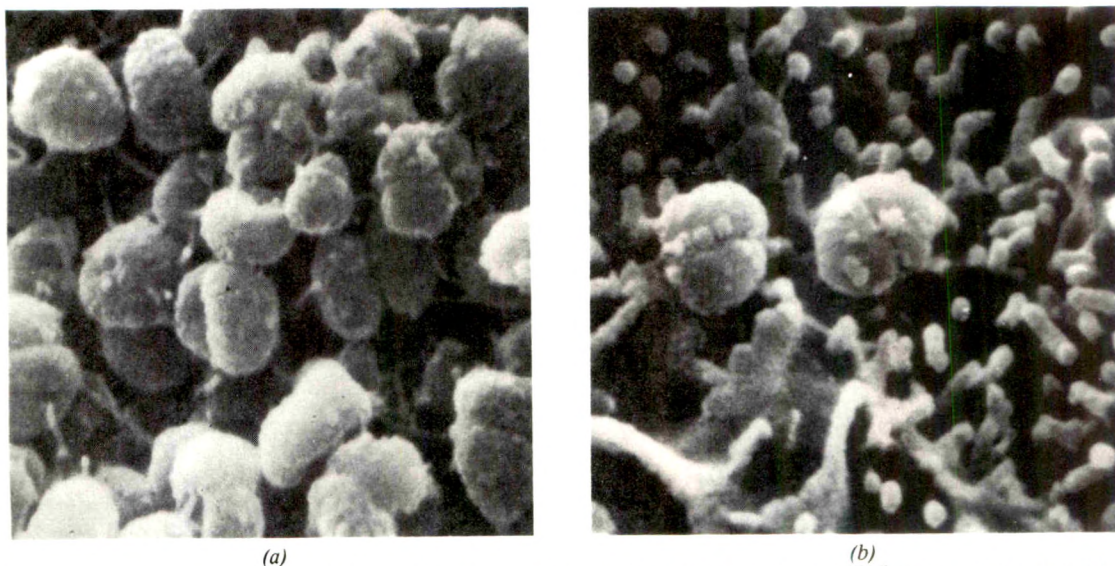


Fig. 4 Scanning electron micrographs of: (a) gonococci (producing T2 colonies) showing diplococcal structure with ragged surface and 'threads' between bacterial cells ($\times 24\,000$ original magnification); (b) buds or protrusions on gonococcal cells producing T2 colonies ($\times 24\,000$ original magnification). (Experimentally challenged organ cultures of Fallopian tube epithelium maintained for 24 hours.)

In organ cultures infected with gonococci of colonial type T3/T4, no notable increase in the number of organisms was observed during the first 30 hours of incubation (Fig. 6).

No living gonococci could be reisolated after 24 hours of incubation of pure organ culture medium when an inoculum containing the same number of organisms was used to infect the organ cultures (Fig. 6).

In all cultures infected with gonococci producing T1/T2 or T3/T4 colonies, the gonococci reisolated from the organ culture media invariably produced colonies on the primary isolation plate which were of the same morphological type as the organisms used to infect the cultures. The aerobic and anaerobic control cultures performed daily on blood agar plates for detection of contaminants in the organ culture media all gave negative results, except for two

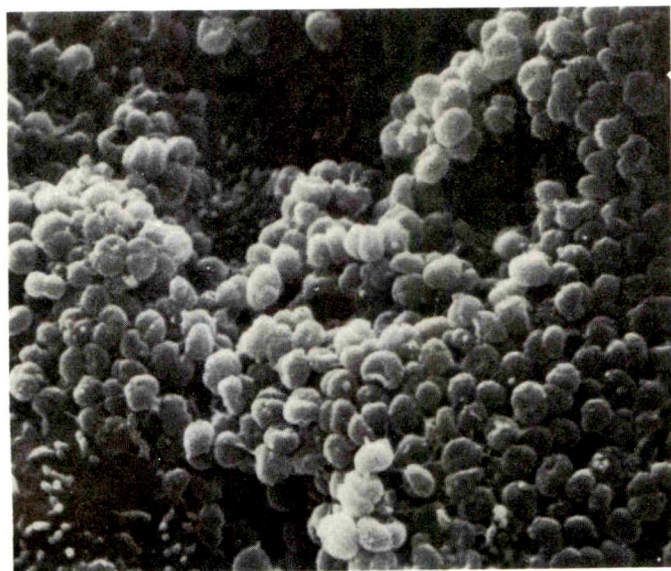


Fig. 5. Scanning electron micrograph of micro-colony of gonococci producing T4 colonies on the surface of Fallopian tube epithelium maintained as organ culture for 24 hours showing 'naked' appearance of the bacterial cells. ($\times 12\,000$).

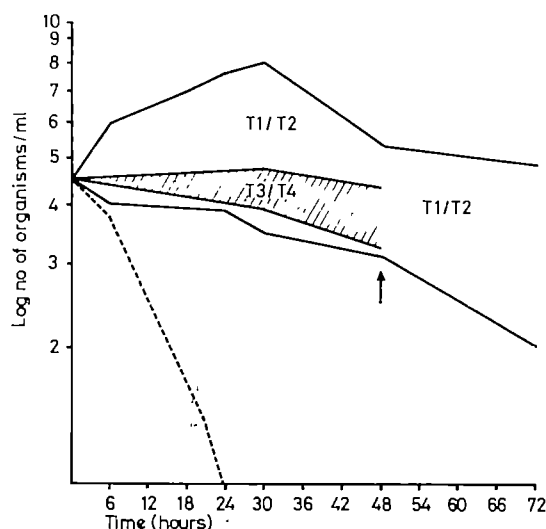


Fig. 6. Number of colony-forming units of gonococci reisolated from the maintenance medium of Fallopian tube organ cultures experimentally infected with approximately 5×10^4 organisms per ml medium of gonococci producing T1/T2 and T3/T4 colonies. Arrow denotes time of replacement of organ culture medium by fresh sterile medium. The broken line (- -) shows the recovery of *N. gonorrhoeae* from pure maintenance medium inoculated with an equal number of T2 colony-producing gonococci.

experiments, the results of which are not reported here.

Discussion

The light beam reflex method reproduces mucociliary wave movements accurately (Toremalm *et al.*, 1974). Variations in the ciliary beat frequency produce corresponding changes in the MCW pattern (Håkansson and Toremalm, 1965). The method has been used for quantitative determination of the ciliary activity in human Fallopian tubes maintained as organ cultures (Weström *et al.*, 1977). In premenopausal women the MCW frequency did not vary during the menstrual cycle and in different parts of the tubes.

Compared to earlier methods used to assess the ciliary activity in tubal organ culture specimens (McGee *et al.*, 1976), the light beam reflex method has several advantages. It offers reproducible numerical values of the MCW activity. Thus the measurements are not dependent on subjective estimations. It also allows rapid registrations and can be performed on any part of the tubal epithelium.

In the present study only specimens from the ampullary portion of the tubes were used, mainly

because they were more easily dissected out than other parts of the tube. Registrations were made from six different areas of the epithelium on each specimen. In each registration, the equipment used covered about 50 ciliated cells. The values of the MCW frequencies were the mean of these six determinations. The deviation from the mean in each of the six registrations never exceeded 50 cycles/min.

The reference values for the MCW frequencies were derived from about 1500 registrations. Only tubal specimens in which the non-challenged controls showed frequencies within the reference range were used. As previously evaluated (unpublished data), the presence of the two antimicrobials added to the culture medium did not affect the MCW frequencies.

In the non-challenged Fallopian tube organ cultures the MCW pattern showed a slow decrease from a mean of 1161 to 894 c/min during the first 54 hours of culture maintenance. The decrease was most probably not due to a reduction in the number of beating cilia, since MCW frequencies in areas of tubal epithelium with a low ciliary density (isthmus) and maintained as organ cultures for an equally long period of time did not differ from those of areas with a high ciliary density (infundibulum) (Weström *et al.*, 1977). The decrease in the MCW frequencies in the non-challenged specimens during culture maintenance corresponded approximately to observations by McGee *et al.*, (1976), who determined the ciliary activity in the same type of organ cultures under a light microscope. The gradual decrease in ciliary activity might be explained by rheological changes in the secretion layer covering the ciliated surface. The sudden cessation of ciliary activity at frequency of about 600 c/min after more than 54 hours of culture maintenance might be due to a depletion of mitochondrial ATP in the ciliary cells.

In organ cultures infected with gonococci producing T1/T2 colonies, the reisolation studies showed a rapid multiplication of the organisms during the first 30 hours of culture maintenance. Corresponding studies on the laboratory-adapted strains producing T3/T4 colonies showed that in no case did the number of cfu/ml exceed that present in the medium at the start of the experiment. Using light microscopy with oblique illumination, the colonial morphology of reisolated gonococci was the same as that of the organisms used to infect the cultures.

SEM showed that gonococci of all the strains studied seemed to become attached to the epithelial surface to form micro-colonies, although they never attached to ciliated cells. Gonococci producing T1/T2 colonies had buds or protrusions on the cell surface and fine threads interspersed between the organisms, as seen in scanning electron micrographs. Such structures were not demonstrable in laboratory-

adapted gonococci. The nature of the structures is not known, but whether or not the threads represent pili is worth consideration.

In this study, no morphological changes of the surface epithelium, including the ciliated cells, were shown by SEM during the 54 hours of culture maintenance. When using Fallopian tube organ cultures experimentally infected with *N. gonorrhoeae* (producing T4 colonies), Taylor-Robinson *et al.* (1974) noted that loss of ciliary activity preceded any damage to the ciliary cells.

In the present study, gonococci producing T1/T2 colonies caused a decrease and subsequent cessation of ciliary activity of the tubal epithelium whereas the laboratory-adapted strains did not. McGee *et al.* (1978) recently reported similar findings. These workers also found that cell-free filtrates (Johnson *et al.*, 1977) and gonococcal endotoxin (McGee *et al.*, 1978) affected the ciliary activity of tubal epithelium maintained as organ culture.

In this study, cell-free filtrates of medium from Fallopian tube organ cultures experimentally infected with gonococci producing T1/T2 colonies as well as endotoxin from a laboratory-adapted strain of gonococci had an effect on the ciliary activity similar to that of gonococcal cells producing T1/T2 colonies. The difference in the effects on the MCW frequencies in organ cultures challenged with whole gonococcal cells and those challenged with endotoxin from the same T4 colony-producing strain may be due to only small amounts of endotoxin being released from the bacterial cells. In contrast to the T1/T2 colony-producing gonococci, this laboratory-adapted strain did not multiply as indicated by the reisolation studies, which suggests a slow cell turnover and subsequent small release of endotoxin.

Reimar *et al.* (1978) were unable to document any effect of *E. coli* endotoxin on the MCW activity in organ cultures of rabbit tracheal epithelium, even when a concentration as high as 1 mg/ml was used. Their period of observation was only five hours. In our study, *E. coli* endotoxin produced cessation of ciliary activity within six hours in half of the experiments irrespective of whether 0.2 or 2.0 µg/ml was used. In 15 of 16 experiments, no MCW activity had been detected after the organ cultures had been maintained for 30 hours. No gradual decrease in MCW activity was observed, which contrasted with the experiments using *N. gonorrhoeae* endotoxin. In the 40 determinations at various times using gonococcal endotoxin, 25 showed an MCW frequency below the normal range, which was in contrast to only four of 64 determinations using *E. coli* endotoxin. The difference in the effect of the two endotoxins might be dose-dependent but could also be due to a differential activity.

Whether the ciliostatic effect shown by gonococcal and *E. coli* endotoxins in our *in-vitro* study also occurs *in vivo* in the naturally diseased host is not known, but, if so, the endotoxins might promote the adherence of invading organisms to the mucosal lining of the Fallopian tubes.

This study was supported by grants from the Swedish Medical Research Council (16X-4509).

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Secretory antibody response of the cervix to infection with *Neisseria gonorrhoeae*

A. McMILLAN,* G. MCNEILLAGE,† H. YOUNG,‡ AND S. S. R. BAIN§

From the *Department of Sexually Transmitted Diseases, Black Street, Glasgow; the †Department of Venereology, Royal Infirmary, Edinburgh; the ‡Department of Bacteriology, University of Edinburgh; and the §Royal Infirmary, Edinburgh

SUMMARY Cervical secretions from 157 women were examined for antibody against *Neisseria gonorrhoeae* by an indirect immunofluorescent antibody test. Antigenococcal antibody was detected in 73 (97%) of 75 infected women, being of the IgG class in 73 (97%), of the IgA class in 71 (95%), and of the IgM class in 29 (39%). IgM antibody was nearly always associated with infections of less than 15 days' duration. Immunoglobulin G, reactive with *N. gonorrhoeae*, was found in 23 (33%) of 70 non-infected women; of these, 19 had non-gonococcal cervicitis. Neither IgA nor IgM antibodies were detected in these women. Antigenococcal IgA and IgG was found in each of 12 women who had no evidence of infection but were contacts of infected men. Successful treatment resulted in a rapid decline in IgA antibody activity but a more gradual decrease in IgG reactivity.

Introduction

Although there has been considerable interest recently in the local immune system of the female reproductive tract there have been few reports relating to infection with *Neisseria gonorrhoeae* (Tapchaisri and Sirisinha, 1976; Tramont, 1977). Antibody-containing plasma cells are found principally in the lamina propria of the endocervix, there being relatively few cells in the endometrium or uterine tubes (Rebello *et al.*, 1975). Immunoglobulin A is the predominant antibody class elaborated by these cells, whose numbers in the endocervix have been shown to increase during infection (Chipperfield and Evans, 1972).

Quantitatively, IgG is the principal immunoglobulin detectable in the cervical secretions (Tjokronegoro and Sirisinha, 1975), the ratio of the concentration of IgG to that of secretory IgA being about 2:1. Ogra and Ogra (1973) demonstrated, however, that the antibody response of the lower reproductive tract to topically applied inactivated poliovirus was associated with IgA, and most studies of the immune response of the genital tract to infection have mainly concerned this class of

antibody (Waldman *et al.*, 1971; Ackers *et al.*, 1975; O'Reilly *et al.*, 1976).

This paper reports our findings on the local immune response to infection with *N. gonorrhoeae*.

Materials and methods

PATIENTS AND DIAGNOSTIC TECHNIQUES

One hundred and fifty seven women were investigated; 92 attended the Department of Sexually Transmitted Diseases at the Black Street clinic, Glasgow, and 65 attended the Department of Venereology at the Royal Infirmary, Edinburgh.

At both centres a Gram-stained smear of material from the urethra and cervix was examined, and culture specimens were taken from these sites and from the anorectum and oropharynx. Infection was excluded only if three sets of culture specimens taken at weekly intervals from these sites gave negative results.

Material for culture from patients attending the Glasgow clinic was taken on charcoal-impregnated swabs and transported to the laboratory in Stuart's transport medium. The culture medium used was Columbia blood agar (Oxoid) containing vancomycin (2.5 µg per ml), trimethoprim (3.0 µg per ml), and polymyxin (15 units per ml). The mean interval between specimen collection and inoculation on the culture medium was nine hours (range 4-16 hours).

Address for reprints: Dr A. McMillan, Department of Venereology, 67 Black Street, Glasgow G4 0EF

Received for publication 30 November 1978

In the Edinburgh clinic, modified New York City (MNYC) medium (Young, 1978) was inoculated directly and held at 36°C until transfer to the laboratory (mean time interval 2.1 hours, range 30 minutes to 3 hours).

At both centres the identity of suspected colonies of *N. gonorrhoeae* was confirmed by the oxidase reaction, sugar utilisation tests, and direct immunofluorescence.

Patients were divided into three groups.

Group 1

This consisted of 75 women from whom *N. gonorrhoeae* was cultured from at least one site. The mean age of this group was 23.4 years, and the mean number of sexual partners within the preceding three months was 1.6. Eight women had previously been infected with *N. gonorrhoeae*, the mean interval between the presenting and most recent infection being 2.7 years (range three months to six years). As a contraceptive method, 58 used an oral oestrogen-progestogen preparation, three had an intrauterine device fitted, and 14 took no contraceptive precautions.

At the time of the initial visit 39 women were in the first half of the menstrual cycle and 36 in the second half.

Two women had acute Bartholinitis and three acute salpingitis. Patients with uncomplicated gonorrhoea were treated in Glasgow with minocycline in a single dose of 300 mg, and in Edinburgh with ampicillin (2 g) and probenecid (1 g) given orally as a single dose. Complicated infection was treated with doxycycline given orally in a dosage of 100 mg every eight hours for 14 days.

Efficacy of treatment was assessed by microbiological examination of material obtained from the urethra, cervix, rectum, and, if indicated, oropharynx. Cure was assumed only if three sets of tests performed at weekly intervals gave negative results.

No treatment failures were observed during the period of the study.

Group 2

Seventy women (34 in Glasgow and 36 in Edinburgh) who had no evidence of gonorrhoea were studied. These women were not known sexual contacts of infected men, had no past history of infection, and had not received antimicrobial therapy within the preceding three months.

The mean age of this group was 23.6 years and the mean number of sexual partners within the preceding three months was 1.8. Fifty-two used oral contraception, two had been fitted with an intra-

uterine device, and 16 took no contraceptive precautions.

Thirty-seven women were in the first half of the menstrual cycle at their initial visit and 33 in the second half of the cycle.

Twenty-two women had clinical evidence of cervicitis; the cervix was congested and tender on palpation, and there was a marked purulent or mucopurulent discharge from the os.

Group 3

Twelve women attended (seven in Glasgow and five in Edinburgh) as named sexual contacts of men with culturally proved urethral gonorrhoea. There was, however, no evidence of gonococcal infection. These women had not received antimicrobial treatment within the preceding three months.

COLLECTION OF SPECIMENS

Cervical secretions were obtained by gentle aspiration through a sterile polythene capillary tube (chromatography column tubing, internal diameter 1.0 mm, obtained from Pharmacia Fine Chemicals, Uppsala, Sweden) attached to a 5-ml syringe containing 1 ml of sterile physiological saline. Secretions in the tubing were ejected into a sterile container and a drop of 0.1% sodium azide in saline added. Specimens obviously contaminated with blood or giving a positive reaction with Haemastix strips (Ames Co. Ltd, Slough, Bucks) were discarded. The diluted secretions were centrifuged at $400 \times g$ for 20 minutes, and the supernatant stored at -20°C until required.

Cervical secretions were obtained from each patient at the initial clinic visit and, in the case of those infected with gonorrhoea, at seven, 14, and 28 days following treatment. In addition to the initial specimen, secretions from non-infected patients were again sampled 14 days later.

Serum from each patient was obtained at the same time as the cervical secretions.

QUANTITATION OF SECRETORY IgA AND IgG

The concentrations of these immunoglobulins were estimated by radial immunodiffusion using commercially available low-level plates (Hoescht Pharmaceuticals, Hounslow, Middlesex). A secretory IgA standard, prepared from colostrum (Samson *et al.*, 1973) and kindly provided by Dr Brian McClelland (Blood Transfusion Service, Royal Infirmary, Edinburgh) was used in estimating IgA, and a serum IgG standard was used in the determination of IgG.

GNOCOCCAL ANTIGENS

Strain 9 of *N. gonorrhoeae* as described by O'Reilly

et al. (1973), and kindly supplied by Dr D. S. Kellogg (Center for Disease Control, Atlanta, Georgia, USA) and cultured on MNYC medium was used as gonococcal antigen. In addition, secretions from eight women with gonorrhoea were tested against the homologous strain of the organism.

To test the specificity of the antibody reactivity, secretions were tested against *Neisseria meningitidis*, *Neisseria perflava*, *Neisseria catarrhalis*, *Neisseria lactamica*, *Staphylococcus aureus*, *Staphylococcus albus*, *Streptococcus faecalis*, and *Escherichia coli*, cultures of these organisms being obtained from the stock collection of cultures at the Department of Bacteriology at the University of Edinburgh Medical School.

INDIRECT IMMUNOFLUORESCENT ANTIBODY TEST

The performance of the IFA test has previously been described (McMillan *et al.*, 1979). Doubling dilutions of secretions were tested against strain 9 of *N. gonorrhoeae*.

The system of grading fluorescence described by Welch and O'Reily (1973) was used: 4 + indicates brilliant fluorescence of all organisms; 3 +, well-defined fluorescence of all organisms in the field; 2 +, low density fluorescence of at least 75% of organisms; and +, occasional fluorescing organisms.

For the purpose of this study 'undiluted secretions' refers to the supernatant fluid obtained after centrifugation of the suspension of cervical aspirate in saline. The titre was taken as the reciprocal of the highest dilution giving a 2+ result.

ADSORPTION OF SECRETIONS WITH RABBIT ANTI-HUMAN SECRETORY COMPONENT

Secretions were adsorbed with serum from a rabbit immunised against human secretory component (Hoescht Pharmaceuticals, UK) or with normal rabbit serum, as described by O'Reily *et al.* (1976).

Results

IMMUNOGLOBULIN CONCENTRATIONS

The concentrations of total secretory IgA and IgG in the undiluted secretions are shown in Table 1. There was considerable variation in the concentration of each immunoglobulin from patient to patient, but there was no significant difference between the mean concentrations of each group of patients.

IMMUNOGLOBULIN CLASSES OF ANTIBODY REACTIVE WITH *N. GONORRHOEA*

Untreated infected patients

The results obtained in the IFA test, using strain 9 of *N. gonorrhoeae* as antigen, are shown in the Figure.

Table 1 Concentrations of secretory IgA and IgG in the secretions aspirated from the cervix of women with untreated and treated gonorrhoea, with non-gonococcal cervicitis, and with no clinical evidence of cervicitis

Category of patient	No. of patients	Concentration of immunoglobulin (mg/dl)	
		IgA	IgG
		Mean Range	Mean Range
Gonorrhoea			
Untreated	28	2.4 1.1-4.8	2.0 1.0-3.6
7 days after treatment	29	2.0 1.1-3.7	2.3 1.0-4.1
14 days after treatment	30	2.3 1.0-3.8	2.3 1.0-3.9
28 days after treatment	28	2.2 1.0-3.8	2.5 1.3-3.9
Non-gonococcal cervicitis	19	2.3 1.2-4.4	2.2 1.1-4.0
No clinical evidence of cervicitis	29	2.0 1.0-4.1	1.8 1.2-3.7

Antibody reactive with *N. gonorrhoeae* was detected in the cervical secretions of 73 (97%) of 75 women with gonorrhoea. In 71 (95%) of these patients the antibody was of the IgA class.

Cervical secretions from 10 women with untreated gonorrhoea were absorbed with anti-human secretory component. In each case, this resulted in a threefold, or greater, reduction in titre of IgA antibody activity as detected in the immunofluorescent test.

Antigonooccal IgM was found in 29 (39%) of the 75 infected women. This antibody was detected in the cervical secretions of 27% (59%) of the 46 women who had been infected for 14 days or less, but in only two (7%) of 29 who had been infected longer. This difference is statistically significant ($P < 0.05$ by the method of binomial probabilities).

Immunoglobulin G reactive with *N. gonorrhoeae* was detected in 73 (97%) infected women, being the only class of antigenococcal antibody found in two patients.

In addition to examining the secretions using strain 9 as antigen, specimens from eight infected women, attending consecutively, were tested against the infecting strain of the organism. No difference in immunoglobulin class or in antibody titre was observed in the IFA test between secretions tested against strain 9 and those tested against the infecting strain.

There was no qualitative difference in the antibodies detected in the secretions between women who had previously been infected and those who had not, but the small number of patients precluded a quantitative comparison.

Of the three women with acute salpingitis and the two with Bartholinitis, antibody of the IgA and IgG classes reactive with the gonococcus (strain 9 and homologous strain) was detected in the cervical secretions of each patient, but no antigenococcal IgM was detectable.

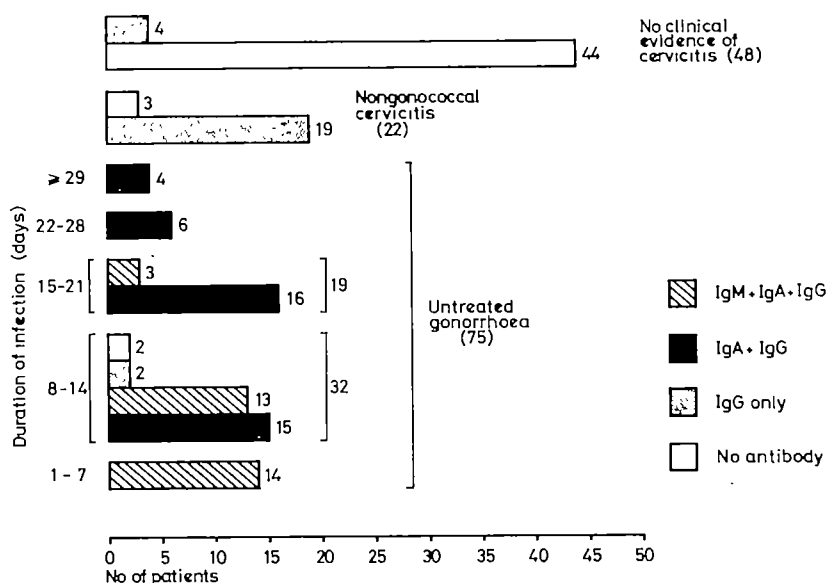


Figure Immunoglobulin classes of antibody reactive with *N. gonorrhoeae* in cervical secretions.

Non-infected women

Antibody of the IgG class reactive with the gonococcus was demonstrated at a titre of 1/2 or less in the secretions of 19 (86%) of 22 women with nongonococcal cervicitis, but in only four (8%) of 48 who had no clinical evidence of cervicitis. Antigonococcal antibody of the IgM or IgA class was not detected in the secretions of this group of women.

Treated patients

The rapid decline in the mean log titre of antigenococcal IgA following successful treatment is shown in Table 2. The mean log titre of IgG antibody declined more gradually.

Within seven days of successful treatment, antigenococcal IgM could not be detected in the cervical secretions of any of the 54 women who

attended at this time. This antibody had been detected in 26 of these patients.

Named sexual contacts not found to be infected

Antibody of the IgA and IgG classes reactive with *N. gonorrhoeae* was detectable in the cervical secretions of each of the 12 patients studied. This antibody was again found when the IFA test was repeated on secretions obtained on two further occasions within the following three weeks. No antigenococcal IgM was found in the cervical secretions of these women.

SPECIFICITY OF ANTIBODIES AGAINST *N. GONORRHOEAE*

The results obtained when cervical secretions from five infected women were examined for antibody against other species of *Neisseria* are given in Table 3. No antibody against these organisms was detectable in the secretions from six non-infected women using monospecific anti-human IgA.

Antibody of the IgG class reactive with *N. meningitidis* group B was found in the secretions of two non-infected women, of group C in two, of group D in one, of group E in two, of group X in one, and of group Z in three. This antibody of this class was also found against *N. lactamica* in five of six of these women, against *N. catarrhalis* in each of the six, and against *N. perflava* in four.

Antibody against *Staph. aureus*, *Staph. albus*, *Strep. faecalis*, and *E. coli* was not detectable in the secretions from these infected and non-infected women.

Table 2 Mean log titre of antibody reactive with *N. gonorrhoeae* in the cervical secretions before and after successful treatment

	No. of patients	Mean log titre of anti-gonococcal antibody	
		IgA	IgG
Before treatment	75	0.682	0.727
7 days after treatment	54	0.273	0.368
14 days after treatment	47	0.064	0.231
28 days after treatment	43	0.000	0.252

Table 3 Antibody against *Neisseria* species in the cervical secretions of five women infected with *N. gonorrhoeae*

Species of <i>Neisseria</i>	Results of IFA test*									
	Case 1		Case 2		Case 3		Case 4		Case 5	
	IgA	IgG	IgA	IgG	IgA	IgG	IgA	IgG	IgA	IgG
<i>N. gonorrhoeae</i>	4+	3+	4+	+	3+	3+	4+	+	3+	3+
<i>N. meningitidis</i>	—	—	—	—	—	—	—	—	—	—
Group A	—	—	—	—	—	—	—	—	—	—
Group B	—	—	—	—	—	2+	—	—	—	2+
Group C	—	—	—	—	—	—	—	—	—	—
Group D	—	—	—	—	—	2+	—	—	—	—
Group E	—	2+	—	—	2+	2+	—	2+	—	—
Group 29 E	—	—	—	—	—	—	—	—	—	—
Group W135	—	2+	—	—	—	—	—	—	—	—
Group Y	+	—	—	—	—	—	—	—	—	—
Group Z	2+	—	—	—	—	2+	—	—	—	+
<i>N. catarrhalis</i>	—	2+	—	—	—	—	—	2+	—	—
<i>N. lactamica</i>	2+	2+	—	2+	—	2+	2+	2+	—	2+
<i>N. perflava</i>	—	—	—	—	—	—	—	—	—	—

* Fluorescence graded as follows: 4+ = brilliant fluorescence of all organisms in a microscopic field; 3+ = well defined fluorescence; 2+ = low intensity fluorescence of all organisms; + = occasional fluorescing organisms; — = no fluorescence.

Discussion

In this study antigenococcal antibody was detected in the cervical secretions of 97% of women with untreated gonorrhoea.

Antigenococcal antibody reactivity was associated with both IgG and IgA regardless of the duration of the infection. IgM antibody was, however, detectable mainly during the first two weeks of infections, thereby resembling the serum antibody response previously reported (McMillan *et al.*, 1979). It is difficult to determine whether this class of antibody is derived from serum or is locally produced; certainly IgM-containing plasma cells may be found in the endocervix (Chipperfield and Evans, 1972).

Tapchaisri and Sirisinha (1976), using strain F62T1 of *N. gonorrhoeae* as antigen, found antibody in the secretions of about 60% of infected women. They further demonstrated that almost all antibody activity was associated with IgG, antigenococcal IgA and IgM being found in only 10% and 5% of patients respectively.

The increased sensitivity noted in the present study may reflect the choice of antigen in the IFA test. Strain 9 of *N. gonorrhoeae* has been shown to share antigenic features with other strains of the organism (O'Reilly *et al.*, 1973).

The threefold or greater reduction in titre of antigenococcal IgA after absorption with anti-human secretory component suggests that most of this antibody is locally produced.

Although antibody of the IgA or IgM classes reactive with *N. gonorrhoeae* was not detected in the cervical secretions of women who had no evidence of gonorrhoea, and who were not known contacts of infected men, antigenococcal IgG was found at a low titre in just under 40% (26/70) of these patients. In

86% of patients with clinical evidence of cervicitis, this antibody was detectable and was probably derived from transudation of natural serum IgG through inflamed mucous membranes. Similar findings were recorded by Tapchaisri and Sirisinha (1976).

Tramont (1977) found IgA reactive with strain 9 of the gonococcus in one of two women who had acute pelvic inflammatory disease but no specific IgA antibody against the infecting strain. Antibody of the IgA class against strain 9 was detected in each of three women in the present study who had acute gonococcal salpingitis. It is difficult to be certain that *N. gonorrhoeae* was the aetiological agent in these women, as other organisms such as *Chlamydia trachomatis* may be present concomitantly (Mårdh *et al.*, 1977).

The rapid decline in IgA antibody activity in the secretions following successful treatment of gonorrhoea had previously been observed in a smaller series of patients by O'Reilly *et al.* (1976). Antigenococcal IgG persisted in the secretions of most patients for at least one month after treatment, resembling the serum IgG response (McMillan *et al.*, 1979).

It was of considerable interest to note that antigenococcal antibody could be detected in the secretions of 12 women who were known sexual contacts of infected men but from whom *N. gonorrhoeae* could not be isolated. There was no history of antimicrobial therapy within the preceding three months, and none of the patients was in an occupation with ready access to such drugs. It is possible that these patients had been infected, but their intrinsic immunity had prevented colonisation by the organism. It is well recognised that only a proportion, up to about 70%, of female sexual

contacts of men with urethral gonorrhoea will be found to be infected (Wallin, 1974).

This project was supported by a grant from the Biomedical Research Committee of the Scottish Home and Health Department (Grant No. K/MRS/36/C38) whose financial assistance is gratefully acknowledged.

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Evaluation of urethral culture for *Neisseria gonorrhoeae* in the routine investigation of men attending a STD clinic

ALEXANDER McMILLAN AND RICHARD S. PATTMAN

From the Department of Sexually Transmitted Diseases, Black Street, Glasgow

SUMMARY Urethral gonorrhoea was diagnosed by culture in 957 (18·8%) of 5076 new male patients attending a sexually transmitted diseases clinic; the infection was asymptomatic in 38 (4%) of these patients. The diagnosis in only six (0·3%) out of 2159 men with urethral gonorrhoea but with no symptoms or signs of urethritis would have been missed if urethral cultures had not been performed.

Introduction

Urethral gonorrhoea in men usually produces symptoms of urethral discharge and dysuria of varying severity. About 15% of men with urethral infection, however, have no symptoms and constitute an important reservoir of infection (Moore *et al.*, 1973; Portnoy *et al.*, 1974; Nielsen *et al.*, 1975).

We decided to examine the value of urethral culture as part of the investigation of all men attending a sexually transmitted diseases clinic.

Materials and methods

All men attending this department between 1 January 1977 and 31 March 1978 were investigated. During this period 5076 new male patients attended.

A careful history was taken from each patient, particular attention being paid to the presence and duration of urethral discharge and dysuria and to whether or not the patient was a sexual contact of another patient with gonorrhoea. The duration of infection was determined from the sexual history of the patient and of his sexual partner.

The urethral meatus was inspected after gentle massage of the penile urethra from the bulb forwards, and a Gram-stained smear of any exudate was examined microscopically. Smears were only prepared if a discharge was present or if the patient was a sexual contact of an infected partner.

Address for reprints: Dr A. McMillan, Department of Sexually Transmitted Diseases, 67 Black Street, Glasgow G4 0EF

Received for publication 20 December 1978

In every case material from the urethra was cultured for *Neisseria gonorrhoeae*. Specimens were taken using charcoal-impregnated bacteriological swabs and sent to the laboratory in Stuart's transport medium. The culture medium used was Columbia blood agar (Oxoid) containing vancomycin (2·5 µg/ml), trimethoprim (3·0 µg/ml), and polymyxin (15 units/ml). The identity of suspected colonies of *N. gonorrhoeae* was confirmed by the oxidase test and sugar utilisation reactions.

After specimens had been taken for bacteriological investigation, the urine was examined macroscopically by the two-glass urine test. The presence of haze, specks, or threads in the urine was noted, as was the period during which the patient had not passed urine before attending the clinic.

Results

Urethral gonorrhoea, as diagnosed by culture, was found in 957 (18·9%) of 5076 men.

The results of Gram-stained smears of exudate from 2903 men with urethral discharge are given in the Table. Urethral gonorrhoea was diagnosed by microscopy and culture in 875 (94·1%) men and by culture alone in 55. Gram-negative diplococci, morphologically resembling the gonococcus, were observed in smears from 67 men; *N. gonorrhoeae* was not, however, isolated on culture. Eleven of these infected men had no symptoms of urethritis, but discharge was noted on examination.

Microscopical examination of urethral material showed presumptive gonococci in 10 of 14 other culture-positive men who were named contacts and had no symptoms or signs of urethritis.

The other 13 patients found to have urethral gonorrhoea had no symptoms of urethritis and no obvious urethral discharge was noted on examination, although most had passed urine within the preceding two hours (mean interval between the time of most recent micturition and that of examination was 95 minutes; range, 30 minutes to 4 hours). Threads, haze, or specks were detected in the urine of seven of these men; microscopical examination of the threads or specks was not performed. These patients attended for treatment of some other condition, such as genital warts or balanoposthitis, and were not known contacts. Three of these 13 men attended during the prepatent period—two to four days (Mahoney, 1940)—of the infection. Of the remaining 10 men, the mean duration of infection was 64 days (range 15-300 days).

Table Results of examination of Gram-stained smear of exudate from patients with urethral discharge

Smear results	Culture for <i>N. gonorrhoeae</i>	
	Positive	Negative
Typical organisms		
Present	875	67
Absent	55	1906
Total	930	1973

In this series, the incidence of asymptomatic infection—that is, lack of symptoms of urethral discharge or dysuria or both—was 4% (38 of 957 men with gonorrhoea). If urethral culture had not been performed routinely on 2159 patients who had no symptoms or signs of urethritis and who were not named contacts, the diagnosis would have been missed in six (0.3%) infected men.

Discussion

Examination of a Gram-stained smear is only moderately sensitive in diagnosing urethral gonorrhoea in symptomatic patients; our finding of 94% sensitivity agreed broadly with that of Jacobs and Kraus (1975). Recent micturition reduces the likelihood of finding gonococci on a Gram-stained smear, although it does not interfere with cultural diagnosis (Judson *et al.*, 1977). This finding may partly explain the disparity between microscopy and culture results.

Failure to isolate *N. gonorrhoeae* from the urethra of 67 men in whom organisms morphologically resembling the gonococcus were observed in Gram-stained smears of exudate may be explained by the inability of the organisms to survive in Stuart's transport medium. Coliforms may grow in this medium and may inhibit growth of *N. gonorrhoeae*

(Amies, 1967). About 3% of gonococcal strains will not grow in medium containing 3 µg/ml of vancomycin (Reyn, 1969), and this may partly explain why the organism was not isolated in these men.

Microscopical examination of a Gram-stained smear is, however, a very insensitive means of detecting gonorrhoea in the absence of clinical signs of urethritis (Handsfield *et al.*, 1974), since culture of material from the urethra is required to establish a diagnosis. Handsfield *et al.* (1974) also demonstrated that culture of material from the anterior urethra was more sensitive than culture of prostatic fluid.

The importance of asymptomatic (that is, without symptoms of urethral discharge and dysuria) but infectious men with urethral gonorrhoea has previously been stressed (Blount, 1972), and most physicians are now aware of this when examining male contacts of infected individuals.

Although Thatcher *et al.*, (1969) found no case of urethral gonorrhoea among 505 military personnel undergoing routine medical examination, Handsfield *et al.* (1974) reported an incidence of asymptomatic infection of just under 1% in 2628 servicemen returning from south-east Asia.

There have, however, been few reports on the prevalence of asymptomatic urethral infection in the civilian population. Carpenter and Westfield (1940) found a prevalence of about 1% in sexually active male prisoners. In a small series, Pariser *et al.* (1964) reported a prevalence of 4% of men attending a venereal disease clinic of their own accord. John and Donald (1978) found that of 203 cases of urethral gonorrhoea 17% had no symptoms and that in 7% the symptoms were so mild as to be ignored by the patient. This contrasts with our finding of a prevalence of 4% in men.

In the present series, although 38 infected men were asymptomatic, 11 had a frank, urethral discharge, and culture would in any case have been performed, as it would have been in the 14 named but asymptomatic contacts. Finding threads or specks in the urine would normally have prompted further investigation, the patient being asked to return for investigation of an early morning smear. The value of this examination has recently been confirmed (Simmons, 1978). In only six infected men (0.3% of patients who had no symptoms or signs of urethritis and who were not named contacts) would the diagnosis have been missed if urethral culture had not been a routine investigation of all clinic patients.

Ideally, where laboratory facilities permit, urethral culture should be performed on each patient routinely; where facilities are limited it would, however, be difficult to justify the screening of every male patient.

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Gonorrhoea in women in Scotland

Evidence of cohorts having a higher or lower incidence than expected

C. B. S. SCHOFIELD

From Newcastle General Hospital, Newcastle-upon-Tyne

SUMMARY Incidences of genital gonorrhoea in women higher or lower than expected occurred in patients aged 15-19 years four years after similar findings in those aged 10-14 years. This pattern was followed five years later in those aged 20-24, 11 years later in those aged 25-34, and 20 years later in those aged 35-44 years. Thus, cohorts of women at greater or lesser risk of acquiring gonorrhoea appear to exist. Most cohorts with a high incidence could be identified when in the 10-14 age group. Especially high rates of infection are predicted in the 25-34 age group in the mid-1980s and in the 35-44 age group in the late 1980s. These findings will affect the timing of health education on sexually transmitted diseases in schools and will demand a high degree of awareness among general practitioners, gynaecologists, and those working in family planning and well-women clinics of the possibility of gonorrhoea occurring in women in these age groups.

Introduction

In a review of sexually transmitted diseases in Scotland between 1972 and 1975 Schofield and McNeil (1977) found a sustained increase in the reported incidence of genital gonorrhoea in women aged 20-24 years (from 333 to 393 per 100 000). Later Schofield (1977) warned that a cohort of promiscuous women born in the mid-1950s might exist. Schofield and McNeil (1977) also noted that over the same period the reported incidence among girls aged 15-19 years had decreased (from 309 to 250 per 100 000). It was, therefore, surprising that in 1976 the incidence rose to 290 per 100 000.

Further study of the incidence of genital gonorrhoea by age groups showed that four years previously in 1972 the incidence among girls under 15 years had risen to 2 per 100 000 compared with 1 per 100 000 for the years 1970, 1971, 1973, and 1974. If the average age of the 15-19 age group was 18 years (it was 17.8 years in Glasgow), and most girls under 15 were aged 14, the possibility of a cohort at special risk of acquiring gonorrhoea had to be considered. For the years 1967 to 1970 the reported incidence of genital gonorrhoea among girls aged 15-19 had risen from 100 to 185 per 100 000, five years before the rise

already occurring in the 20-24 age group. This led to further examination of the reported incidence of genital gonorrhoea and of the population by age groups of women in Scotland for the years 1950 to 1977 inclusive.

Methods

The annual number of cases of genital gonorrhoea by age groups were obtained from the Scottish summaries of the quarterly returns (ISD(D)5) submitted by the special clinics to the Scottish Home and Health Department. The age groups 35-44 years and 45 and over were not separated in the quarterly returns until 1956, and the age groups 15-19 and 20-24 years not until 1966. The annual number of cases within the age groups before separation nationally have been estimated from attendances at the Glasgow clinics, where such information was available from 1950 onwards and trends closely followed those in Scotland as a whole.

Information on the number of women by age in Scotland from 1950 to 1977 was kindly provided by the Registrar General for Scotland (personal communication). The annual incidence of genital gonorrhoea in women by age groups is reported in rates per 100 000. The incidence in women aged 45 and over was so low (2.3 per 100 000) that they have not been included. Instead of rates per 100 000 of those under 15 years being used as published the

Address for reprints: Dr C. B. S. Schofield, Ward 34, Newcastle General Hospital, Westgate Road, Newcastle-upon-Tyne, NE4 6BE

Received for publication 15 September 1978

more realistic rates per 100 000 of those aged 10-14 are quoted. (Discussion with colleagues showed that none of the cases occurred in patients under 10 years.)

Results

In addition to the rate of six per 100 000 noted in the 10-14 age group in 1972, similarly high rates occurred in this age group in 1954, 1958, and between 1967 and 1969 (Fig. 1). They were followed at intervals by similar peaks in all the age groups—four years later in those aged 15-19, nine years later for those aged 20-24, and 15 years later for those aged 25-34. In the 15-19 age group high rates in 1951 and 1953 were followed by high rates in the subsequent age groups at the same intervals as above and 20 years later in the 35-44 age group while high rates between 1966 and 1968 preceded those in the 20-24 age group from 1971 to 1973 and in the 25-34 age group in 1977.

Lower rates of increases less than in the adjacent years were noted in the 15-19 age group in 1959 (four years after a rate of only one per 100 000 in the 10-14 age group) 1964, and 1969 and were followed by similar patterns in the subsequent age groups. Low rates also occurred in the 25-34 age group in 1955 and in the 35-44 age group in 1964.

The years when a higher and when a lower incidence of genital gonorrhoea than expected in the various age groups has been recorded or is projected up to 1990 are shown in Tables 1 and 2 respectively.

Estimates of variation in caseload require a knowledge of the size of population at risk in addition to the incidence. The years when large or small populations have moved through the age groups or are projected to do so are shown in Table 3. In Scotland the postwar 'bulge' of female live births was greatest in 1948 and has increased the population of the age groups as follows: those aged 10-14 in 1961 (13 years ahead), those aged 15-19 in

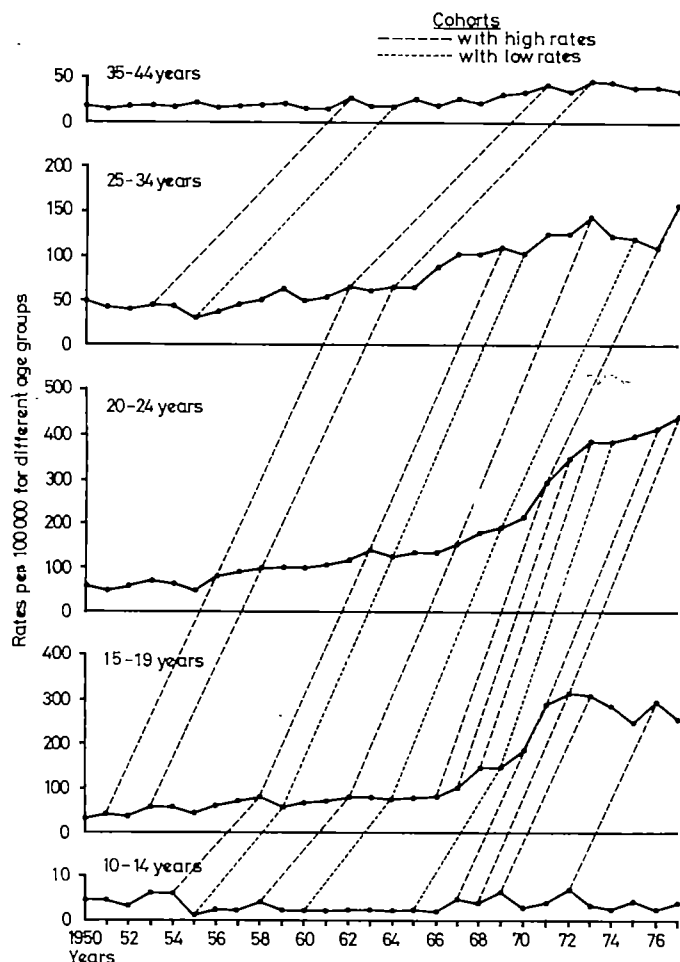


Figure Genital gonorrhoea in women in Scotland (1950-77).

Table 1 *Years of recorded and projected higher incidences in the age groups*

Years of birth	Years of higher incidence in the age groups				
	10-14	15-19	20-24	25-34	35-55
1924				1953	1962
1933			1956	1962	1971
1935		1951	1958	1964	1973
1940	1954	1958	1963	1969	1978
1944	1958	1962	1967	1973	1982
1948 }		1966 }	1971	1977	1986 }
1950 }		1968 }	1973	1978	1988 }
			1976 }	1979 }	
1953 }	1967 }	1971 }	1977	1982	
1955 }	1969 }	1973 }	1978	1984	
1958	1972	1976	1981	1987	
1961	1975	1979	1984	1990	

Table 2 *Years of recorded and projected lower incidences in the age groups*

Years of birth	Years of lower incidence in the age groups				
	10-14	15-19	20-24	25-34	35-44
1926				1955	1964
1941	1955	1959	1964	1970	1979
1946	1960	1964	1969	1975	1984
1951	1965	1969	1974	1980	1989
1962	1976	1980	1985		

Table 3 *Years of recorded and projected large and small populations in the various age groups*

Years of birth	Population size	Age groups				
		10-14	15-19	20-24	25-34	35-44
1922	Large				1954	1964
1939	Small	1952	1957	1962	1971	1981
1948	Large	1961	1966	1971	1980	1990
1952	Small	1965	1969	1974	1983	
1964	Large	1977	1981-2	1986-7		

1966 (18 years ahead), and those aged 20-24 in 1971 (23 years ahead). Thus from the study of previous trends, the increase in population will occur in the 25-34 age group in 1980 (32 years ahead) and in the 35-44 age group in 1990 (42 years ahead). A previous large population was born about 1922 and a small one in 1939. Between 1948 and 1952 the number of live births fell by 13% while in the following 12 years to 1964 the number rose by 17%. A steady decline in the number of live births occurred between 1964 and 1976 but was less pronounced the following year, so that by 1977 there were 35% fewer births than in 1964.

Discussion

Irrespective of whether the incidence of genital gonorrhoea in women was higher or lower than expected, the intervals between the increase or decrease becoming apparent in age groups was always four years between those aged 10-14 and 15-19 years, five years between the 15-19 and 20-24 age groups, six years between the 20-24 and 25-34 age groups, and nine years between those aged 25-34 and 35-44 years. This points to cohorts of women at greater or lesser risk of acquiring gonorrhoea during their years of sexual activity.

Most cohorts could be identified as early as in the 10-14 age group. Cohorts of women with high rates followed six of the 10 occasions when there was an incidence of 4 or more per 100 000 in those aged 10-14 years while one cohort with low rates followed the occasion when the incidence was only 1 per 100 000. Thus, by this time these girls will have been exposed to influences whose effects would govern their sexual lives. What these influences are, why their effects should differ almost from one year to the next, and when they affect the cohort is not as yet apparent, but they certainly warrant further investigation and research. These findings will influence the time when health education on sexual intercourse and sexually transmitted diseases should be provided in schools. Possibly children require education on their sexual responsibilities even in primary school or at the latest in the first two years of secondary education. One cohort with a high incidence rate was not detected until the girls were aged 15-19 years between 1966 and 1968 and may have been subject to different influences at a later date, but the high incidence has continued into the 25-34 age group in which it can be predicted to produce a rate of about 200 per 100 000 by 1979.

The times taken for large or small populations to move from one age group to the next differed from those taken for groups with high or low rates of genital gonorrhoea, possibly reflecting differences in the amount of sexual activity in the various age groups.

From considerations of the combined effects of the projected variations on the incidence of genital gonorrhoea in women (expressed as rates per 100 000) and changes in population size by age, it is possible to predict when relatively large or small numbers of

cases of gonorrhoea will occur over the next decade or so and within which age groups they will fall.

The number of cases of gonorrhoea within the 10-14 age group is predicted to fall steadily until 1990 at least, while that in the 15-19 age group can be expected to rise to a maximum in 1979 and then decline by 1982; the incidence will then decline for a least 12 years. The number of cases in the 20-24 age group are likely to increase considerably until 1984, especially in 1978 and 1981; after 1984 it will fall moderately until 1987 and thereafter more sharply until 1990. The number of cases of genital gonorrhoea in the 35-44 age group can be expected to rise in 1978 then fall sharply until 1981. From then on a pronounced increase in the number up to 1986 to 1988 will occur, after which there will be a moderate decline until 1990.

If these predictions are confirmed and there is an increased incidence of gonorrhoea in the various age groups in the years ahead they will have implications for those providing health care as well as for those working in special clinics. General practitioners, gynaecologists, those working in well-women and family planning clinics, and others concerned with primary medical care will need to have a high index of suspicion of the possibility of gonorrhoea occurring in the older age groups as well as in teenagers and those in their early 20s, already recognised as being at special risk. In the 1980s those aged 25-44 will also be at special risk.

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How often are gonorrhoea and genital yeast infection sexually transmitted?

R. N. THIN,* PAULINE RENDELL,* AND JANE WADSWORTH†

From the *Department of Genital Medicine and the †Computing Unit, St Bartholomew's Hospital, London

SUMMARY Although gonorrhoea is often regarded as the sexually transmitted disease against which others are measured, its infectivity is not clearly understood. Estimates of the infection rate have varied from 5-90%. In this study, 50 couples with gonorrhoea were matched with 50 couples with genital yeast infection. Gonorrhoea was diagnosed in both partners of 32 couples and genital yeast infection in both partners of 21 couples. These figures provide an indication of the sexual transmission of these conditions. The higher figure for gonorrhoea may be related to a greater urgency in tracing contacts.

Introduction

Gonorrhoea is often regarded as the sexually transmitted disease against which others are measured. Yet its infectivity is not clearly known. For example, the infection rate among female contacts of men with gonorrhoea was 91% as assessed by Thin *et al.* (1971), but at the same hospital Barlow *et al.* (1976) indicated that it was only 66%. Furthermore, Lee *et al.* (1972) suggested that infectivity of gonorrhoea was very much lower, between 5 and 40% with a mean of about 27%.

In view of the variation in these figures and the lack of precision in their meaning, we decided to conduct a case control study to compare the prevalence of infection among contacts of patients with gonorrhoea and the contacts of patients with genital yeast infection.

Patients and methods

All patients with gonorrhoea were urged to bring their sexual contacts to the clinic for examination. Eighty-two consecutive pairs of male and female partners attending this department within 28 days of each other were included in the study.

All female patients with a documented recurrence of vaginal yeast infection, or with a history of more than two episodes during the preceding six months, were urged to bring their regular partner(s) to the

clinic. All men with penile yeast infection were also urged to bring their regular partner(s). Eighty-three consecutive pairs of male and female partners attending within 35 days of each other were included in the study.

DIAGNOSTIC TECHNIQUES

The methods used to diagnose gonorrhoea in men and women have already been described (Alani *et al.*, 1977; Thin and Shaw, 1979). The methods used to diagnose genital yeast infection in women were as described by Thin *et al.* (1975), but only positive Gram-stain and culture results will be considered here. In men with a suspected penile yeast infection and in all male contacts, a swab was either taken from any sub-preputial exudate or rolled around the coronal sulcus in the absence of exudate elsewhere; this swab was used to prepare a smear for Gram staining and a Sabouraud culture was inoculated. Typical Gram-stain appearances or cultural findings or both were regarded as positive results (Thin *et al.*, 1975).

Statistical comparisons were made using a χ^2 test with Yates's correction, or Fisher's Exact Test for contingency tables, or McNemar's test for differences between affected partners.

Results

DEMOGRAPHIC DATA

Fifty couples with gonorrhoea were matched with 50 couples with genital yeast infection for age, race, and marital state. In the group with yeast infection, the women's ages ranged from 16 to 35 years with a

Address for reprints: Dr R. N. Thin, Department of Genital Medicine, St Bartholomew's Hospital, London EC1A 7BE

Received for publication 23 January 1979

mode of 26 and a mean of 24.2 years, and the men's ages ranged from 17 to 54 years with a mode of 29 and mean of 27.4 years. In the group with gonorrhoea the women's ages ranged from 16 to 38 years with a mode of 23 and a mean of 24.7 years, and the men's ages ranged from 16 to 52 years with a mode of 28 and a mean of 27.0 years. In each disease group 46 couples were white, two were Negro, and two couples were Asian; both partners of all couples were of the same racial origin. In the group with gonorrhoea, three men had two partners but only one of them, and both his partners, were in the matched group. Each patient with yeast infection had only one partner.

OVERALL RESULTS

The overall results of matched and unmatched couples with gonorrhoea and genital yeasts are shown in Table 1. Of the patients with gonorrhoea, 62.2% of both partners were affected but only 41% of both partners had genital yeasts. If the findings in the matched pairs are examined alone a significant difference is noted ($\chi^2 = 14.25$; $P < 0.001$) (Table 2). This difference is examined further in Table 3, which shows that in couples with gonorrhoea significantly more are both infected than in couples with genital yeast infection.

TIME-INTERVAL BETWEEN EXAMINATIONS

More men than women had gonorrhoea and more women than men had genital yeasts (Table 1). The figures in Tables 4 and 5 support this finding.

The difference in the infection rates might be due to the difference in the interval between examination of the individual members of each pair. Among matched pairs with gonorrhoea, 86% were examined within 14 days of each other and in 60% both had positive results (Table 6). Similar findings were obtained when those examined within seven days of each other were compared with the remainder.

In contrast, only 74% of matched pairs with genital yeast infection were seen within 14 days of each other and both partners had positive results in only 28% (Table 7).

Table 2 Comparison of results for infected men and women (50 matched couples)

Infection	With positive results		
	Men and women	Men only	Women only
Yeast infection	21	6	23
Gonorrhoea	32	12	6

The proportion of infected men and women are significantly different in the two disease groups.

$$\chi^2 = 14.25 \quad P < 0.001$$

Table 3 Comparison of results for matched couples in which both partners were affected

Infection	With positive results	
	Men and women	Men or women
Yeast infection	21	29
Gonorrhoea	32	18

In gonorrhoea significantly more couples are both infected.

$$\chi^2 = 4.01; \quad P < 0.05$$

Table 4 Comparison of results for matched couples with only one infected partner

Infection	With positive results	
	Man	Woman
Yeast infection	6	23
Gonorrhoea	12	6

In gonorrhoea significantly more men are infected while significantly more women have yeasts.

$$\chi^2 = 8.08; \quad P < 0.01$$

Table 5 Comparison of results for matched couples

Results for men	Results for women			
	Gonorrhoea*		Yeast infection†	
	+	-	+	-
Positive	32	12	21	6
Negative	6	0	23	0

+ positive - negative

* $\chi^2 = 1.39$ (not significant) by McNemar's test

† $\chi^2 = 8.83$; $P < 0.01$ by McNemar's test

Table 1 Overall results

Results for partner		For gonorrhoea				For yeast infection			
Male	Female	Matched couples	Unmatched couples	Total		Matched couples	Unmatched couples	Total	
				No.	%			No.	%
+	+	32	19	51	62.2	21	13	34	41.0
+	-	12	6	18	22.0	6	6	12	14.5
-	+	6	7	13	15.8	23	14	37	44.5

+ positive - negative

Table 6 Interval between examination of both partners for gonorrhoea

Examination at (days)	Positive results for	
	Both partners	Only one partner
0-14	30	13
15-28	2	5

* Fisher's Exact test $P < 0.05$

Table 7 Interval between examination of both partners for yeast infection

Examination at (days)	Positive results for	
	Both partners	Only one partner
0-14	14	23
15-28	7	6

 $\chi^2_1 = 0.46$ not significant

Discussion

Case control comparisons tend to have bias, and the main ones in our study were: (a) all patients with gonorrhoea were urged to bring all partners (although all men with a penile yeast infection were urged to bring regular partners, only women with recurrent yeast infection were urged to bring regular partners); (b) contact-tracing was more vigorous in patients with gonorrhoea; and (c) gonorrhoea spreads via a chain of infection while genital yeast infection can arise in a stable partnership.

In addition, we considered that accurate data could not be collected on the time interval between the infecting sexual intercourse and examination or on the number of exposures between each patient and an infected partner. We believe, however, that our findings do provide some measure of infectivity in an STD clinic population.

Thus, under the conditions influencing the behaviour of clinic patients and the design of the study, gonorrhoea (64%) appears to be transmitted significantly more often than genital yeast infection (42%). The figure for gonorrhoea resembles the figure of 66% for infected female contacts quoted by

Barlow *et al.* (1976) and is notably higher than the estimate given by Lee *et al.* (1972). The figure for genital yeasts closely resembles the estimate of 39% reported by Thin *et al.* (1977). Gonorrhoea infects men more often than women but genital yeasts infect women more often than men. The latter findings may be related to autoinfection or reinfection from the bowel (Rohatiner, 1966).

The greater urgency of contact-tracing in gonorrhoea brings sexual contacts to the clinic early and there is a high proportion of positive results (Table 6). The lesser urgency in genital yeast infection brings a slower response from contacts with a lower proportion of positive findings, although differences were not significant (Table 7).

Our findings support the well-established view that gonorrhoea is an infectious disease and that rapid, accurate contact-tracing is important in its control. Contact-tracing is less important in genital yeast infection. The previously reported association of genital yeast infection with other STDs (Thin *et al.*, 1977), however, supports the need for contact-tracing in patients with this condition.

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The need for a chlamydial culture service*

J. R. WILLCOX, P. G. FISK, J. BARROW, AND D. BARLOW

From the Chlamydia Research Laboratory, Rayne Institute, and the Department of Genitourinary Medicine, St Thomas's Hospital, London

SUMMARY In a prospective study of unselected, female patients attending a sexually transmitted disease clinic one in eight patients would have been erroneously declared free of infection in the absence of a chlamydial culture service. *Chlamydia trachomatis* is now accepted as a causative organism of non-specific urethritis and post-gonococcal urethritis in men and non-specific genital infection in women. Thus, facilities for the isolation of *C. trachomatis* should be an essential aid in the management of women attending STD clinics. Male patients would also benefit if such facilities were readily available.

Introduction

Chlamydia trachomatis has become recognised increasingly as a common human pathogen in many disciplines of medicine. The identification of women harbouring *C. trachomatis* is desirable for many reasons. It is now accepted that these women are a potential source of infection to their male partner(s) and that they may develop salpingitis (Mårdh *et al.*, 1977).

In pregnancy there may be premature labour and, after delivery, an increased risk of post-partum pelvic infection in the mother and inclusion conjunctivitis in the child (Rees *et al.*, 1977). Cases of neonatal pneumonitis have also been reported (Beem and Saxon, 1977).

It is regrettable therefore that those few clinics with laboratory facilities for the isolation of *C. trachomatis* tend to be looked on as research centres and are not generally regarded as providing an essential aid to the diagnosis and management of the most common sexually transmitted disease (Department of Health and Social Security, 1976).

In March 1976 a prospective study of female patients attending this department was carried out. Patients were unselected apart from the exclusion of those who had received antibiotic treatment in the previous two months.

*Adapted from a paper presented at the spring meeting of the Medical Society for the Study of Venereal Diseases at Leeds, 19-20 May 1978

Address for reprints: Dr J. Barrow, Lydia Department of Genitourinary Medicine, St Thomas's Hospital, London SE1 7EH

Received for publication 26 October 1978

The main aim of the study was to collect data from patients harbouring *C. trachomatis* for comparison with those patients with other sexually transmitted diseases and those who were free from infection.

Over an 18-month period, information was obtained by means of confidential questionnaires, from which—together with clinical and laboratory findings—we hoped to gain a greater understanding of the epidemiology of chlamydial genital infection.

The data presented in this paper are restricted to symptoms and reasons for attendance in unselected female patients.

Patients and methods

All patients in the study were screened for other sexually transmitted diseases using standard methods (Barlow *et al.*, 1976). Samples were taken from the vagina for microscopy and culture of *Trichomonas vaginalis* and *Candida albicans* and from the urethra, cervix, and rectum (where indicated) for microscopy and culture of *Neisseria gonorrhoeae*. Samples for herpes simplex isolation were taken where clinically indicated. A specimen for cervical cytology and blood for antitreponemal antibodies were also collected.

Specimens for the isolation of *C. trachomatis* were taken from the endocervix (including junctional material) using sterile cottonwool swabs. The specimens were inoculated on to McCoy cell monolayers either immediately or after storage at 4°C overnight. If a longer period was to elapse before inoculation, the samples were kept at -70°C. After the slides had been incubated for 48 hours, *C. trachomatis* was diagnosed by the finding of characteristic brown-staining inclusions after

flooding of the fixed cell-layers with iodine. Full details of this technique are described elsewhere (Reeve *et al.*, 1975).

Results and discussion

CHLAMYDIAL SERVICE FOR WOMEN

During the study over 2000 samples from the female endocervix were processed, and the isolation rate for chlamydiae varied month by month between 20 and 25% (average 23.6%) after exclusion of contaminated specimens.

The symptoms on attendance in three groups of patients are shown in Table 1. Those patients with chlamydial infection alone and those with no sexually transmitted disease (NSD) are compared with patients with gonorrhoea alone (Barlow and Phillips, 1978). There was no significant difference between the presenting symptoms in any of these groups.

The overall isolation rate of *C. trachomatis* in unselected women in our study was similar to that obtained in other studies (Hilton *et al.*, 1974; Oriel *et al.*, 1974; Nayyar *et al.*, 1976; Woolfit and Watt, 1977). Presenting symptoms gave no clue to the presence or absence of chlamydiae, which confirms previous reports (Oriel *et al.*, 1974; Burns *et al.*, 1975; Oriel *et al.*, 1978).

One hundred and seventy-eight (31.4%) chlamydiae-positive patients were contacts of patients with non-specific urethritis (NSU) (Table 2). At many centres these contacts would have received treatment epidemiologically in the absence of a chlamydial service laboratory.

Furthermore, 122 (21.5%) patients were contacts of gonorrhoea. Possibly most of these cases would not have received treatment for their chlamydial infection without a chlamydial service. It is not standard practice for many clinics to issue contact slips to men developing post-gonococcal urethritis (PGU) and thus the treatment of the female contact for post-gonococcal non-specific genital infection (NSGI) is often overlooked—even if she has attended for follow up. Additionally, should a male patient default before PGU can be diagnosed, there is no way of excluding, or treating, NSGI in his partner even on epidemiological grounds.

Over 28% (159) of patients with chlamydial infection came to the clinic of their own accord and 58 were referred by their general practitioners, family planning clinics, or other hospital departments. A further 8% attended because they suspected that their partner had a sexually transmitted disease but were unable to provide any definite information.

Thus, 68.6% of chlamydiae-positive female patients were not contacts of NSU. In 1976, 5747 new female patients attended this department and if a conservative chlamydial isolation rate of 20% is taken it would be expected that 1149 positive isolations would be obtained. Only 361 patients (the contacts of NSU) would have been treated epidemiologically, leaving 788 (68.8%) women who were chlamydiae-positive but would not have been treated. If these results are extrapolated for the whole of England, it can be estimated that in 1976 there were 18 300 women seen in clinics with undiagnosed, and, therefore, untreated, chlamydial infection. Presumably most of these patients were told that they were free from infection.

Table 1 Symptoms on attendance in female patients

Symptoms	Chlamydial infection alone (n = 153)		Gonorrhoea alone (n = 258)		NSD (n = 133)	
	No.	%	No.	%	No.	%
Vaginal discharge	68	44.4	103	40	44	33
Dysuria	13	8.5	30	12	11	8.3
Pruritus	22	14.4	16	6	17	12.8
Abdominal pain	10	6.5	18	7	13	9.8
Frequency	17	11.1	6	2	11	8.3
Other	13	8.4	22	9	11	8.3
No symptoms	60	39.2	124	48	51	38.3

Table 2 Reasons for attendance in 567 chlamydiae-positive female patients

Reason	No.	%
Contact of NSU	178	31.4
Contact of gonorrhoea	122	21.5
Own accord	159	28.1
General practitioner referral	58	10.2
Boyfriend (with possible STD)	45	7.9
Other	5	0.9

CHLAMYDIAL SERVICE FOR MEN

As chlamydiae-positive and chlamydiae-negative urethritis are treated identically in most centres, and since there have been few reports of *C. trachomatis* isolation from the male urethra in the absence of urethritis (Holmes *et al.*, 1975; Alani *et al.*, 1977), at first sight there would seem to be no great advantage in taking routine chlamydial cultures from all male patients.

In two areas, however, chlamydial isolation would be valuable. The first is in cases where double infection with gonococci and chlamydiae have occurred. Traditionally the after-effects of gonococcal urethritis must be eradicated before PGU can be diagnosed. This takes up to two weeks in most centres. If a chlamydial infection could be diagnosed within two or three days of the patient's first attendance treatment could be started earlier, thus reducing the time during which the patient would be under observation—a mutual benefit to patient and

clinic staff. Secondly, it is unlikely that the vexed problems in the diagnosis and management of recurrent nongonococcal urethritis, whether it is due to relapse or reinfection, will be resolved without at least those cases due to chlamydial infection being identified.

Conclusion

A large reservoir of untreated chlamydial infection seems to exist in female patients attending similar departments in the UK. Routine screening tests or clinical findings are unlikely to be of help in such cases (Oriel *et al.*, 1978).

Included among these patients are contacts of gonorrhoea (21.5% in our series), and it has been suggested (Richmond and Oriel, 1978) that all women with gonorrhoea should be given anti-chlamydial treatment since chlamydial isolation rates among such women have been shown to be as high as 30-60% (Hilton *et al.*, 1974; Oriel *et al.*, 1974; Burns *et al.*, 1975; Woolfit and Watt, 1977).

Had we adopted such a policy in this department we would have treated 52.9% of chlamydiae-positive women (including those who were contacts of NSU) but also many gonorrhoea-positive but chlamydiae-negative women.

Encouraging reports of specific tests to detect anti-chlamydial antibody in both serum and local secretions have been published recently (Treharne *et al.*, 1977, 1978) but it remains to be seen how specifically such tests reflect current as opposed to past chlamydial infection.

We believe, however, that a chlamydial diagnostic service—either by direct culture of the organism or by other means—should now be regarded as a necessity rather than a luxury.

This study was supported by a generous grant from the Research Endowments Fund, St Thomas's Hospital.

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Double-blind comparison of two regimens in the treatment of nongonococcal urethritis*

Seven-day vs 21-day courses of triple tetracycline (Deteclo)

I. V. THAMBAR,† P. D. SIMMONS,† R. N. THIN,† S. DAROUGAR,‡ AND P. YEARSLEY‡

From the †Department of Genital Medicine, St Bartholomew's Hospital, and the ‡Virus Laboratory, Institute of Ophthalmology, London

SUMMARY In a double-blind comparison of two regimens of triple tetracycline (Deteclo, Lederle) in the treatment of nongonococcal urethritis, 62 (88·6%) of 70 patients treated with one tablet twice daily for 21 days and seen four weeks after starting therapy had satisfactory results. This was significantly better than the findings among the 73 patients treated with one tablet twice daily for seven days and followed for four weeks, among whom only 47 (64·4%) had satisfactory results. Results were also better for the group treated with the 21-day regimen at three months after the start of treatment. When analysed individually at four and 12 weeks, urethral discharge, urethral Gram-stained smears, and first-glass urine test all gave similar results, which were markedly better than those before treatment. It appears that the longer course of treatment is indicated where any regular partner may not be treated. Slightly fewer patients had satisfactory results among those who admitted consuming alcohol than among those who did not. Chlamydiae-negative patients, treated for seven days, had fewer clinically satisfactory results than other sub-groups.

Introduction

The tetracycline group of drugs appears to be among the most effective agents for treating nongonococcal urethritis (NGU) (Morton 1975; King and Nicol, 1975). Although many tetracycline preparations and dosages have been described few comparative trials have been carried out.

In this report, we describe the results of a double-blind trial of the triple tetracycline, Deteclo (Lederle), one tablet given every 12 hours for seven days, compared with one tablet given every 12 hours for 21 days.

Methods

PATIENTS

The aim was to study an unselected group of men presenting consecutively with nongonococcal

urethritis (NGU). As NGU varies in clinical severity we decided to include only men with a visible discharge containing at least 10 leucocytes per microscopic field in at least 20 fields examined with a $\times 100$ objective and $\times 8$ eyepieces using only one microscope. Patients with a history of hypersensitivity to tetracyclines were excluded as were men taking antacids, milk, or oral iron, patients with a previous history of hepatitis, and patients who were unable to attend for three months' observation after treatment.

INVESTIGATION

Before treatment was started, urethral secretions were collected for Gram staining and microscopy for leucocytes, organisms, and fungal elements. A culture was taken for *Neisseria gonorrhoeae* and a wet preparation was examined microscopically for *Trichomonas vaginalis*. In addition, a urethral swab was taken for culture for *Chlamydia trachomatis*. Urine was examined by the two-glass test. These investigations have been described in greater detail by Alani *et al.* (1977).

DRUG ADMINISTRATION

The study was double-blind, with patients randomly allocated to one of two treatment groups.

*Paper presented in part at the spring meeting of the Medical Society for the Study of Venereal Diseases at Leeds, 19-20 May 1978

Address for reprints: Dr R. N. Thin, Department of Genital Medicine, St Bartholomew's Hospital, London EC1A 7BE

Received for publication 4 January 1979

21-day regimen

Patients in this group were given one Deteclo tablet twice daily for seven days, followed by one identical placebo tablet twice daily for 14 days.

Seven-day regimen

Patients in this group were given one Deteclo tablet twice daily for seven days, followed by one identical placebo tablet twice daily for 14 days.

Patients were instructed not to take the tablets with milk.

OBSERVATION AFTER TREATMENT

Patients were asked to attend one, four, eight, and 12 weeks after starting treatment and to hold their urine for at least four hours before attending. At each visit they were examined for the presence of visible urethral discharge, and material for Gram staining and for microscopy for leucocytes, together with a culture for gonococci, were collected after gentle urethral massage. A urethral culture for *C. trachomatis* was collected and the urine was examined by the two-glass test.

All clinical observations and results of laboratory investigations were entered on a special proforma; only the data recorded before treatment and at four and 12 weeks after the beginning of therapy are reported here.

Retreatment (either because of reinfection or treatment failure) was indicated if:

- (1) Four weeks after starting treatment, a urethral discharge, containing at least 10 leucocytes per microscopic field on Gram stain as already defined, was present;
- (2) At later follow up, urethral discharge containing leucocytes in the Gram stain as defined or haze, specks, or threads in the first urine glass containing leucocytes in the centrifuged deposit in the numbers already defined were present.

Patients without a discharge and with clear first urine were regarded as having a satisfactory outcome.

The effect of treating regular female partners was assessed; regular partners were those with whom the patient had had contact more than twice in the preceding three months (Alani *et al.* (1977).

During the period of the trial, approximately 90% of Gram staining and microscopy was performed by one male nurse, and all slides were kept and checked by one doctor; 90% of urinoscopy was also performed by one observer.

Statistical comparisons were made using Yates's χ^2 test and Fisher's Exact test.

Results

Of a total of 200 patients who started treatment, 57 were excluded for various reasons such as failure to

attend for follow up and finding gonococci on culture. Thus, of the remaining 143 patients, 70 were treated with the 21-day regimen and 73 with the seven-day regimen of Deteclo, all of whom completed four weeks' follow up.

After treatment and follow up and before the treatment code was broken, a general appraisal of outcome was made according to the stated criteria and cases were classified as having a satisfactory outcome or a recurrence (treatment failure or reinfection).

Once the code had been broken, statistical analysis showed that the patients in the two groups resembled each other in age distribution, country of origin, marital state, history of previous urethritis, chlamydial culture results, follow-up pattern, and time urine had been held before initial examination and investigation.

By four weeks after the start of treatment, a satisfactory overall response was found in 89% of cases treated for 21 days and in 64% of cases treated for seven days (Table 1). By twelve weeks after the start of treatment findings were satisfactory in 74% of cases treated for 21 days and in 61% of those treated for seven days (Table 1). The difference in findings at four weeks is statistically significant.

The urethral discharge, urethral smear, and urinary findings on which clinical assessment of recurrence was based are shown in Table 2. A comparison between the findings at four weeks and at 12 weeks with the pre-treatment findings was noteworthy ($P < 0.001$ in all cases). The comparison of findings between the two regimens was less marked ($P < 0.01$ at four weeks in all cases; no significant differences at 12 weeks) as would be expected from the findings in Table 1. Comparison between the numbers of normal and abnormal findings in each parameter with the figures for each of the other two parameters at four and 12 weeks showed no differences.

The relationship of sexual intercourse during treatment or follow up or both to clinical outcome is shown in Table 3. Although the numbers with recurrence after 12 weeks were small, approximately the same proportion of patients admitted sexual

Table 1 Overall results of treatment

Treatment regimen with Deteclo	No. with clinically satisfactory results (out of no. followed up) at			
	Four weeks		12 weeks	
	No.	%	No.	%
21-day	62/70*	88.6	38/51†	75.5
7-day	47/73*	64.3	32/52†	61.5

* $\chi^2_1 = 10.25$, $P < 0.01$

† $\chi^2_1 = 1.44$, $P < 0.25$

Table 2 Detailed findings before and after treatment

Examination	% in each drug-treatment group					
	With urethral discharge		Abnormal Gram-stain		Abnormal first-urine findings	
	7-day	21-day	7-day	21-day	7-day	21-day
Before treatment	94	96	97	100	87	89
At four weeks	29	14	33	11	48	20
At 12 weeks	0	7	4	6	3	14

Table 3 Sexual intercourse during follow up

Clinical outcome	Treatment group	No. admitting intercourse during			
		Weeks 1-4		Weeks 5-12	
		No	Yes	No	Yes
Satisfactory	21 days	34	4	11	22
	7 days	26	5	4	22
Recurrence	21 days	5	1	3	2
	7 days	10	2	2	5

No significant differences

intercourse as among those with a satisfactory outcome.

The effect on results at four weeks of treating regular female partners, who attended this department, was examined and the findings are shown in Table 4. Although few partners attended, the findings suggested that, in the group treated with the 21-day regimen, treatment of regular partners had little effect on the patients' failure rate. Among those treated for only seven days, however, the recurrence rate was significantly higher when the partner was not treated.

The effect of alcohol consumption during the four weeks after starting treatment is shown in Table 5. Alcohol was associated with a slightly higher recurrence rate in both groups.

In Table 6, the outcome at 12 weeks is compared with any past history of urethritis (admitted in the history or recorded in the clinical documents); with both regimens a satisfactory outcome was strongly associated with no previous urethritis and recurrence with a past history of urethritis.

Table 4 Effect of treating regular female partners on findings four weeks after start of treatment

	Treatment group	Satisfactory outcome	Recurrence	
			No.	%
Partner treated	21 days	14	4	22.2
	7 days	14	4	22.2
Partner not treated	21 days	20	4	16.7
	7 days	7	9	56.2

In comparison of results of two regimens when partner not treated $\chi^2 = 5.17$, $P < 0.05$

Table 5 Relation between admitted alcohol consumption and clinical outcome at four weeks

	Treatment group	Satisfactory outcome	Recurrence	
			No.	%
Alcohol	21 days	30	5	14.3
	7 days	27	12	30.8
No alcohol	21 days	26	3	10.3
	7 days	17	6	26.1

No significant differences

Table 6 Relation of outcome at 12 weeks to past history of urethritis

History of urethritis	Treatment group	Satisfactory outcome	Recurrence
Positive	21 days*	10	9
Negative		28	4
Total		38	13
Positive	7 days†	9	12
Negative		23	8
Total		32	20

* $\chi^2 = 5.91$, $P < 0.025$

† $\chi^2 = 3.95$, $P < 0.05$

CHLAMYDIAL CULTURE RESULTS

Positive *C. trachomatis* culture results were obtained before treatment from 16 (23%) patients treated for 21 days and from 12 (16%) treated for seven days (Table 7). Only one patient had a positive culture result after therapy and it is not clear why (Table 7).

The clinical response four weeks after starting therapy in relation to the *C. trachomatis* findings

Table 7 *Chlamydial culture results before and after treatment*

Treatment group	Positive culture results (in relation to treatment)			
	Before	Four weeks after		12 weeks after
	No. %	No.	%	
21 days	16 22.9	0		0
7 days	12 16.4	1	2.5	0

before the start is shown in Table 8. No relation was evident between outcome and chlamydial culture result in the group treated for 21 days; in the group treated for seven days a higher recurrence rate occurred in the culture-negative patients than in the culture-positive patients.

Table 8 *Clinical response in relation to chlamydial culture results before treatment*

Treatment group	Outcome at four weeks	Chlamydial culture result before treatment		Probability
		+	-	
21 days	satisfactory	5	21	0.661
	recurrence	2	9	
7 days	satisfactory	8	17	0.067
	recurrence	1	14	

+ positive - negative

Discussion

The results indicate that a better cure rate can be expected after 21 days of treatment than after seven days of treatment. The results with the 21-day regimen, however, were not as good as the 20-day regimen with oxytetracycline reported by John (1971); the seven-day regimen gave similar results to the five-day regimen with oxytetracycline described by John (1971). Although he (John, 1971) was working in this department, his methods and criteria were different.

Unfortunately, like so many other studies of the treatment of NGU, there was a high default rate but this was similar in the two groups; this may be one reason for the lack of significant difference between the results from the two treatment groups at 12 weeks. Results of studies in different departments cannot be closely compared. Our follow-up rate was worse than in some studies (Bhattacharyya and Morton, 1975) but better than others (Willcox, 1968).

The improvement in urethral discharge, urethral smear, and first urine findings appeared sooner in patients treated for 21 days than in those treated for seven days (Table 2). By 12 weeks, however, the response was similar in the two groups. Morton and

Read (1957) and Fowler (1970) observed that recurrence was more common in patients treated with tetracycline than in those given a placebo. It is interesting to speculate on these differences. Perhaps the short course fails to eliminate causative micro-organisms or their antigens and allows better development of natural immunity which produces improvement. Critical analysis of these findings supported the preliminary overall general assessment. We had wondered if comparison of each parameter with the others might show differences indicating one which was more or less sensitive, but all appeared to give similar findings.

Opinions vary concerning the need for prolonged follow up. Our findings support the need for this even after 21 days of treatment.

Patients are usually advised to avoid intercourse during antimicrobial treatment and for a period afterwards (King and Nicol, 1975). In our study, recurrence and further intercourse were not related, (Table 3) although this may have been because we did not differentiate between treatment failure and reinfection. This contrasts with the report by Perera (1975). Despite the present findings, and until more equally critical studies have been undertaken, it appears wise to continue to advise patients to abstain from sexual intercourse during, and for a while after, treatment.

It is in doubt whether or not partners of men with NGU should be treated (Bhattacharyya and Morton, 1973; Evans, 1977). Although the numbers were small, our findings (Table 4) showed that among those whose partners were not treated patients given the seven-day regimen had a significantly higher recurrence rate than patients given the 21-day regimen. Thus, when there is doubt if a partner can be traced and treated a 21-day course of treatment should be prescribed.

It is also common practice to advise avoidance of alcohol (King and Nicol, 1975); Perera (1975) found a link between recurrence and alcohol consumption. The higher recurrence rate associated with alcohol consumption in our study (Table 5) supports this view, and although differences were not significant it appears wise to advise patients to avoid alcohol.

The correlation between recurrence and a past history of urethritis (Table 6) is in agreement with the findings of Morton and Read (1957) but contrasts with those of Evans (1977, 1978). Evans (1977, 1978), however, excluded patients treated for NGU during the year before his study.

It is interesting that the recurrence rate among the chlamydiae-negative cases treated for seven days was higher than in the other group (Table 7). This is in keeping with the findings reported by Handsfield *et al.* (1976) from Seattle, USA.

The main conclusions to be drawn from this study are that Deteclo, one tablet given twice daily for 21 days, gives better results than one tablet given twice daily for seven days. The longer course should be given when any regular partner may not be traced and treated.

We thank Dr W. Atia and Mr Wilson for their help in the clinic and Mrs Jamieson and Mrs Wadsworth for analysing the data. Tablets used in this study were kindly supplied by Cyanamid Ltd. The chlamydial laboratory work was supported by grants from the Department of Health and Social Security and an anonymous donor.

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Rapid staining technique for *Trichomonas vaginalis*

Preliminary report

M. J. BALSDON, N. GREEN, C. W. ANDREW, AND D. H. JACKSON

From the Department of Genitourinary Medicine, Royal South Hants Hospital, Southampton

SUMMARY A simple, rapid, staining method for the identification of *Trichomonas vaginalis* has been tested on cultured trichomonads and specimens of vaginal discharge. Fifty-eight stained slides of vaginal discharge were examined and trichomonads were correctly identified in the 31 specimens from patients with confirmed vaginal trichomoniasis. No false-positive results were obtained. This staining procedure could prove a useful addition to wet-film and cultural methods.

Introduction

Trichomoniasis is a common cause of genitourinary symptoms in women and may be responsible for some cases of nongonococcal urethritis in men. Usually diagnosis in women is easily made by examination of a specimen of vaginal discharge diluted in isotonic saline on a slide using phase-contrast or darkground microscopy. A carefully prepared and examined wet film is considered to give as accurate results as cultural methods (Morton, 1976). In a few cases in women (Gardner and Kaufman, 1969a) and in most cases in men (Candiani *et al.*, 1973) the trichomonads are not easily recognisable because they are few, non-motile, and in an encysted form.

Staining methods for the recognition of *Trichomonas vaginalis*, such as Papanicolaou or Giemsa, are not of use in routine clinical settings because they are too time-consuming (Gardner and Kaufman, 1969b). The recognition of *T. vaginalis* in Gram-stained smears was described by Cree (1968), but this method was not considered of use by Thin and Michael (1970).

This preliminary report describes a simple, rapid, staining method for trichomonads which can be used routinely in a department of genitourinary medicine.

Materials and methods

The staining method used is based on the Diff-

Quik*, a rapid differential stain set developed for use in haematology.

To develop the stain for detecting *T. vaginalis* it was initially used on cultured trichomonads until optimal staining results were obtained. Specimens of vaginal discharge from patients were then examined. A platinum-loop specimen of discharge (or Feinberg-Whittington culture medium containing trichomonads) was mixed on a degreased slide with a drop of saline and spread thinly. It was then air-dried in warm conditions and stained.

STAINING TECHNIQUE

The slide was dipped consecutively in the following reagents of the Diff-Quik set:

- (1) Arylmethane dye, 0.002 g/l in methanol (eight dips of one second each);
- (2) Xanthene dye, 1.25 g/l in phosphate buffer, pH 6.60 (five dips of one second each);
- (3) Thiazine dye, 1.25 g/l in phosphate buffer, pH 6.60 (five dips of one second).

The slide was then washed in phosphate buffer, pH 7.0, and blotted dry. The whole procedure took two to five minutes, depending on the drying process and the thickness of the smear. The slide was then examined microscopically using an oil immersion objective ($\times 500$ or $\times 1000$ magnification).

PATIENTS

Fifty-one women were studied, aged 17 to 38 years; they were of English origin except for three from the West Indies. Twenty-seven women were selected because vaginal trichomoniasis was suspected on

Address for reprints: Dr M. J. Balsdon, Department of Genitourinary Medicine, St Mary's Hospital, Portsmouth, Hants

Received for publication 18 December 1978

*Harleco, Herstal (Liège), Belgium

Table Results of staining of specimens of vaginal discharge

Diagnosis	Diff-Quik stain			Wet film (positive results)			
	+	—	Total	Alone	With culture	Culture alone	Total
Trichomoniasis	31	0	31	4	26	1	31
Other	0	27	27				
Total	31	27	58				

+ positive —negative

clinical grounds, and in 31 specimens from 24 of these women trichomonads were identified on wet film or culture or both. Twenty-seven specimens were obtained from a further 24 women selected as controls. These women did not have trichomoniasis but attended either as contacts of men with non-specific urethritis, or because they had candidosis or genital warts, or for examination to exclude disease. Thus, 58 specimens of vaginal discharge were examined from the 51 women studied.

Results

After preliminary trials to obtain optimal results, 30 smears of cultured trichomonads were stained by the

technique described. Similar stains were carried out on the 58 specimens of vaginal discharge. These were examined microscopically by a technician who had no previous knowledge of the diagnosis. The results are shown in the Table. All the known cases of vaginal trichomoniasis were identified by the Diff-Quik staining method. Up to two minutes' microscopy was allotted to each stained slide, but stained trichomonads were recognised in most known cases in the first few fields. We were unable to identify the trichomonads in Gram stains of the same material with any consistency.

The trichomonad was readily identified by its light-blue cytoplasm, red flagellae and axostyle, and purple oval nucleus in the forward part of the

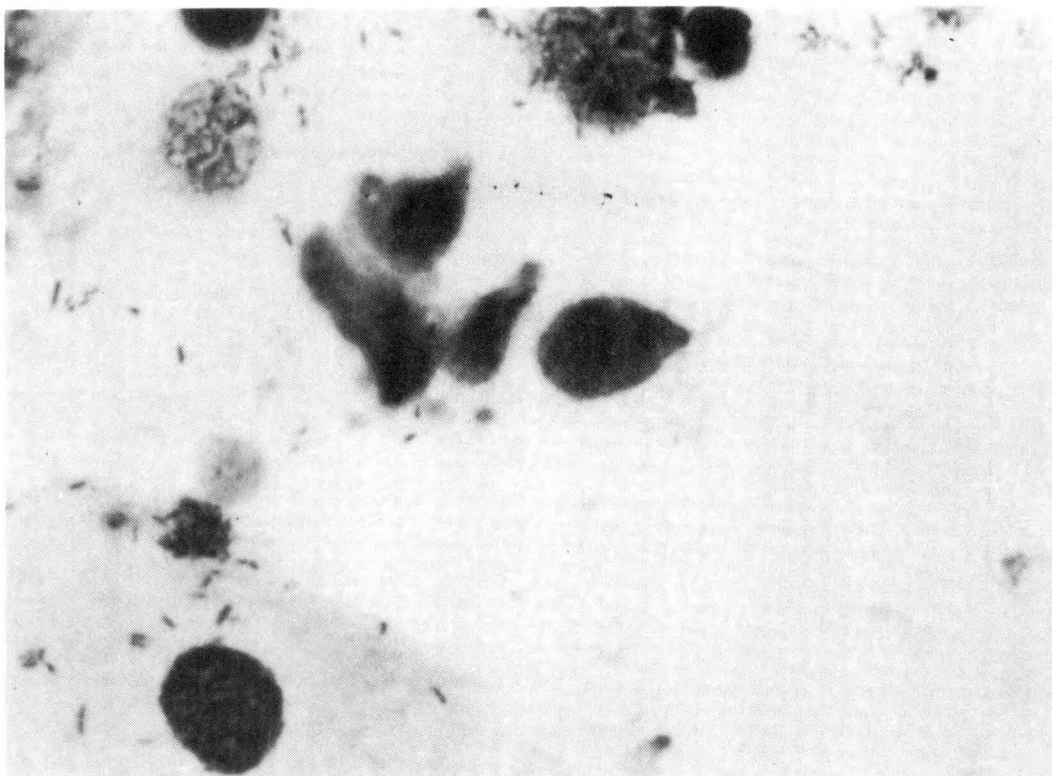


Fig. 1 Four trichomonads with epithelial cells and a polymorph (upper right centre). The four flagellae and undulating membrane of one trichomonad are clearly shown (Diff-Quik stain of vaginal discharge).



Fig. 2 Four trichomonads with six polymorphs and cellular debris. The lower-right trichomonad clearly shows the nucleus, cytoplasm, axostyle, flagellae, and undulating membrane (Diff-Quik stain of vaginal discharge).

protozoon (Figs. 1 and 2). The undulating membrane was usually also seen and stained red. Even in encysted forms the flagellae and nucleus were readily identified, thus enabling differentiation from epithelial and white blood cells.

Cultured trichomonads were also easily recognised after being added to specimens of urethral discharge taken from six men with non-specific urethritis.

Discussion

This preliminary report shows that trichomonads can be easily identified by this staining method. All known cases of trichomoniasis were positively identified and one case (female) was diagnosed even when wet-film methods and initial culture gave negative results. The results are comparable with those of the Giemsa method but are more rapidly obtained.

The stain should prove useful in women with features suggesting trichomoniasis but in whom no trichomonads are identified on the wet film. (The wet film itself can be stained, thus obviating the need for further samples). Other patients who would be suitable for investigation by this method could include women with inflammatory cervical smears of

unknown origin and men with non-specific urethritis—particularly those cases which are recurrent and unresponsive to routine therapy. Smears of urethral and prostatic material or urinary sediment could be used in the latter cases. The method could prove useful as an adjunct to cultures for trichomoniasis, particularly for patients seen in general practice, where smears as well as cultures could be sent to the laboratory.

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False-positive test results for syphilis in relatives of a patient with systemic lupus erythematosus

SUSAN JESSOP AND PHYLLIS BOTHA

From the Departments of Dermatology, Rheumatology, and Bacteriology, University of Cape Town and Groote Schuur Hospital, South Africa

SUMMARY In a family of nine members, two had systemic lupus erythematosus and seven positive serological test results for syphilis. None of the affected subjects had a history or physical signs of syphilis, but two had positive results to the *Treponema pallidum* immobilisation test. The explanation for these findings is not known, but possibly they were all false-positive reactions.

Introduction

Familial aggregation of systemic lupus erythematosus (SLE) is widely reported and has been reviewed by Masi (1968), who found an incidence of 1-2% in first-degree relatives of patients with SLE. The reason for this finding is not clear, but it is probable that both genetic and environmental factors play a role. Humoral abnormalities are found in about 10% of first-degree relatives, the most common being hypergammaglobulinaemia, rheumatoid factor, antinuclear antibody, and false-positive lipid test results for syphilis (Morteo *et al.*, 1961.)

We report a family in which two members had SLE and seven of the other eight had positive results to serological tests for syphilis, including the fluorescent treponemal antibody test (FTA-ABS) and the *Treponema pallidum* haemagglutination (TPHA) test.

Material and methods

SEROLOGY

The Venereal Disease Research Laboratory (VDRL) test followed a standard method (Communicable Disease Center, 1969). The FTA-ABS test was performed as described by Hunter *et al.* (1964) and the TPHA test as described by Johnston (1972).

PATIENTS

Case 1

A 50-year-old woman presented in 1974 with a large leg ulcer of 27 years' duration. She was found to have

thinning of the scalp hair, discoid lupus erythematosus, polyarthritis, and mouth ulcers. On investigation her haemoglobin was 11.2 g/dl, white cell count $2.7 \times 10^9/l$, with normal hepatic and renal function. The LE-cell test gave a positive result, the antinuclear antibody was present at a titre of 1/500, the VDRL test gave a positive result at a titre of 1/2, and the FTA-ABS test gave a positive result with a homogeneous pattern. Her symptoms improved with rest in bed and the administration of salicylates. She remained well until May 1977, when she was admitted in a stuporose state and died, probably of cerebral lupus.

Case 2

The 29-year-old daughter of Case 1 presented in 1977 with severe haemolytic anaemia, positive results to the LE-cell test, and antinuclear antibody at a titre of 1/100.

Because of our interest in familial aggregation of lupus, we then examined the siblings of Case 2. Our findings are summarised in the Table. None of the subjects had a history or physical signs suggesting a connective tissue disease or syphilis, but seven of the eight tested showed some abnormality in the serological test results for syphilis. Two subjects had a positive result to the *Treponema pallidum* immobilisation (TPI) test. None had received recent antibiotic therapy.

Discussion

The family which we describe is not unusual in having two members with SLE. The occurrence of false-positive reactions in family groups is less well known and has been reviewed by Putkonen and Lassus (1965), who reported two families with an

Address for reprints: Dr S. Jessop, Department of Dermatology, Groote Schuur Hospital, Cape Town, South Africa

Received for publication 12 October 1978

Table Results of serological tests in siblings of Case 2 for SLE and syphilis

Sibling	Year of birth	Test results					
		ANA	CH ₅₀	VDRL	FTA-ABS	TPI	TPHA
1	1925	1/500	119	1/1	ND	ND	+
2	1947	—	149	—	B	ND	—
3	1948	1/100	70	—	—	6%	—
4	1949	—	137	—	B	ND	—
5	1950	—	172	1/1	B	55%	+
6	1954	—	175	—	—	ND	—
7	1957	—	147	—	+	ND	+
8	1959	—	154	—	B	9%	+
9	1961	—	172	1/8	+	93%	+
10	1966	—	161	1/1	+	8%	+

+ positive — negative

ANA = antinuclear antibody

CH₅₀ = total haemolytic complement (normal 160-210)

ND = not done

B = borderline

aggregation of false-positive reactors, one of the affected subjects having rheumatoid arthritis. Kostant (1972) later described a further two families, including one in which three generations were affected. Tuffanelli (1968) examined 103 false-positive reactors and their 199 relatives and found a high incidence of antinuclear antibody, rheumatoid factor, and hypergammaglobulinaemia. He suggested that this implied an inherited defect in immunoglobulin control mechanisms, and this could help to explain the familial aggregation of both false-positive reactors and SLE.

It seems possible that the family we describe has a hereditary abnormality associated with false-positive serology and a predisposition to develop SLE, the abnormal serology being a marker of the 'lupus diathesis'. A study (Lowenstein and Rothfield, 1977) of related and unrelated household members showed a higher incidence of antinuclear antibody in related persons but more household members with positive immunofluorescent findings on skin biopsy. This supports the view that both genetic and environmental factors could be concerned in the pathogenesis of SLE.

The serological tests for syphilis (Jaffe, 1975) fall into two groups, the treponemal and the non-treponemal. The latter, including the VDRL test, use non-specific cardiolipin as antigen and may give a positive result in a wide range of conditions, including viral illnesses, malaria, leprosy, and the connective tissue disorders. In a study of 110 chronic false-positive reactors, Johansson (1971) found that 30 had probable or definite SLE and 25 were completely normal. It was initially thought that the treponemal tests were specific for syphilis, but this has not proved to be the case, 15-23% of patients with SLE having a false-positive result to the FTA-ABS test (Shore, 1976). In SLE this reaction may take the form of a beaded (Kraus *et al.*, 1970) or a

homogeneous fluorescence, the latter being indistinguishable from the true positive (Shore and Faricelli, 1977). The atypical, beaded pattern appears to be due to the presence of anti-DNA antibody in the serum (Kraus *et al.*, 1971) but the factor responsible for the homogeneous FTA-ABS test has not been identified. The TPHA test may be even less specific in some laboratories. Garner *et al.*, (1973) applied the test to 274 subjects with false-positive reaginic test results but negative FTA-ABS test results, and found 11.3% to give positive results to the TPHA test.

The TPI test is thought to be highly specific for syphilis, although its results may become negative if the patient is treated early in the disease (Lassus, 1968). Moore and Mohr (1952), examining a group of people thought to be false-positive reactors, found 16% to have positive results to the TPI test. They concluded that clinical judgement was probably at fault rather than the test, but they were, of course, unable to prove this.

The positive results to the TPI test in two of our subjects raise the question of possible false-positive results to this test. The only recognised false-positive reaction is that seen when the patient is taking an antibiotic, but this was not the case in our subjects. Another possibility, which cannot be completely excluded, is that two members of the family had had syphilis and four others had false-positive results to serology. Both venereal and endemic syphilis commonly occur in the small rural communities of South Africa (Du Toit, 1969). Du Toit found, however, that nasopharyngeal ulceration, mucous patches, condylomata lata, gummata, and bone lesions were common in endemic syphilis, and our subjects had none of these.

The most likely explanation for our findings is that the members of the family described above had a variety of serological abnormalities which gave rise to

false-positive results to the VDRL, FTA-ABS, TPHA, and TPI tests in certain members and to SLE in two cases.

We are indebted to N. Richardson and H. J. Koornhof, of the South African Institute for Medical Research, for the TPI tests, to Dr D. Whitelaw for assistance in collecting blood samples, and to Dr H. Brown and Professor W. Gordon for access to their patients. One of the authors (SJ) is in receipt of a grant from the Arthritis Foundation, Cape.

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Social distribution of sexually transmitted diseases

A survey of female clinic registrations

P. M. BACON

From the Department of Social Administration, University of Hull

SUMMARY Social and medical data on 1672 female patients attending a venereal disease clinic were recorded over a four-year period. Analysis of these data provided a demographic and social profile of female clinic attenders and indicated considerable variations in patterns of diagnosis between different social categories of patients. The probability of diagnosis of different infections varied according to age, marital state, social class, employment status, and previous record of attendance.

Introduction

Surveys of patients attending venereal disease clinics at the Hull Royal Infirmary were undertaken during the period 1970-74 to investigate the social distribution of sexually transmitted diseases on Humberside. An earlier paper presented an analysis of a three-year sample of male clinic registrations (Heywood and Bacon, 1975). The results of this study are derived from a survey of female registrations during the period from 1 January 1970 to 31 December 1973.

Patients and methods

All new, post-pubertal cases presenting in UK residents during the survey period were included. Cases presenting in foreign residents visiting the UK for periods of up to one month were excluded. Personal and medical data, including the patient's age, marital state, occupational background, suspected source of infection, medical and sexual history, and conditions diagnosed were recorded for each new case registered. Registrations were categorised under five diagnoses: syphilis, gonorrhoea, non-specific genital infection, nil, and 'other conditions'; 'other condition(s)' refers to conditions requiring treatment in the clinic. All data were processed for computer analysis, which was carried out with the aid of an SPSS programme in the University of Hull computer centre.

Results

AGE DISTRIBUTION

The age distribution of the total number of registrations is shown in the Figure. The rapid upturn leading to a sharp peak in the late teens was followed by an almost equally rapid decline in numbers during the early 20s when the marriage rate is at its highest. The median age of patients was 21.5 years.

AGE AND MARITAL STATE

The distribution of registrations by age and marital state is shown in Table 1. The high proportion of registrations in the separated/divorced category among patients aged 25 and over is noteworthy.

Of the 387 cases presenting in married women living with their husbands, the husband was cited as the suspected source of infection in 277 (72%) cases. Husbands were also cited in 23 cases presenting in separated wives. Twenty-six cases among patients in the age range 15-22 who were pregnant at the time of examination are included in the number given in the single/childless category (Table 1).

DIAGNOSES

The annual distribution of diagnoses and the overall pattern is given in Table 2. The total number of diagnoses of gonorrhoea (628) included approximately 15-20% which were diagnosed on epidemiological evidence only.

AGE AND DIAGNOSIS

The distribution of diagnoses in different age groups is shown in Table 3. More than one-third (35.8%) of

Address for reprints: P. M. Bacon, Department of Social Administration, University of Hull, Hull, HU6 7RX

Received for publication 30 November 1978

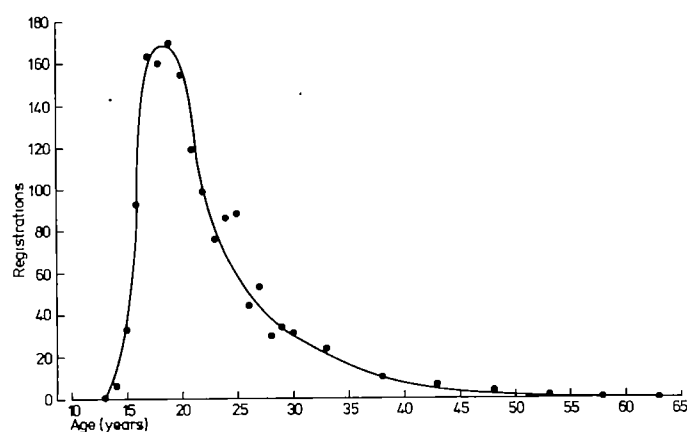


Figure 1 Age distribution of 1672 female registrations (1970-3)

Table 1 Age and marital state

Age	Marital state										Total no. of registrations	% of total registrations
	Single, childless		Unmarried mother		Married		Separated or divorced		Widowed			
	No.	%	No.	%	No.	%	No.	%	No.	%		
<20	538	85.9	28	4.5	32	5.1	28	4.5	0	0	626	37.4
20-24	278	52.1	55	10.3	122	22.8	79	14.8	0	0	534	31.9
25-29	42	17.0	16	6.4	105	42.3	81	32.7	4	1.6	248	14.8
30-39	15	7.5	9	4.5	89	44.7	81	40.7	5	2.5	199	11.9
40-49	1	2.0	2	3.9	31	60.8	16	31.4	1	2.0	51	3.1
50+	1	7.1	0	0	8	57.1	2	14.3	3	21.4	14	0.8
Total	875	52.3	110	6.6	387	23.1	287	17.2	13	0.8	1672	100.0

Table 2 Registrations and diagnoses by year (1970-73)

Year	Diagnosis										Total no. of registrations
	Syphilis		Gonorrhoea		NSGI		Other condition(s)		Nil		
	No.	%	No.	%	No.	%	No.	%	No.	%	
1970	11	3.7	118	39.6	31	10.4	38	12.7	111	37.2	298
1971	8	1.9	167	39.0	52	12.1	48	11.2	166	38.8	428
1972	4	0.8	173	36.5	85	17.9	50	10.5	174	36.7	474
1973	1	0.2	170	36.0	55	11.6	45	9.5	210	44.5	472
Total	24	1.4	628	37.6	223	13.3	181	10.7	661	39.5	1672

Table 3 Age and diagnosis

Age	Diagnosis										Total no. of registrations
	Syphilis		Gonorrhoea		NSGI		Other condition(s)		Nil		
	No.	%	No.	%	No.	%	No.	%	No.	%	
<20	6	1.0	225	35.9	73	11.7	91	14.5	254	40.6	626
20-24	6	1.1	205	38.4	86	16.1	49	9.2	200	37.4	534
25-29	7	2.8	115	46.4	31	12.5	20	8.1	81	32.7	248
30-39	5	2.5	70	35.2	31	15.6	16	8.0	81	40.7	199
40 +	0	0	13	20.0	2	3.1	5	7.7	45	69.2	65
Total	24		628		223		181		661		1672

SOCIAL CLASS AND DIAGNOSIS

MARITAL STATE AND DIAGNOSIS

EMPLOYMENT STATUS AND DIAGNOSIS

Patterns of diagnosis in relation to the employment status of patients are shown in Table 7. All those describing themselves as 'unemployed' or as a 'housewife' were assigned to these categories, which included patients in all marital state categories.

Marital state	Diagnosis										Total no. of registrations	
	Syphilis		Gonorrhoea		NSGI		Other condition(s)		Nil			
	No.	%	No.	%	No.	%	No.	%	No.	%		
Single, childless	9	1.0	272	31.1	119	13.6	116	13.3	380	43.4	875	
Unmarried mothers	3	2.7	50	45.4	14	12.7	10	9.1	36	32.7	110	
Married	8	2.1	161	41.6	66	17.0	35	9.0	129	33.3	387	
Separated/divorced	4	1.4	139	48.4	24	8.4	18	6.3	111	38.7	287	
Widowed	0	0	6	46.1	0	0	2	15.4	5	38.5	13	
Total	24		628		223		181		661		1672	

[illegible]Table 6 *Patterns of incidence of gonorrhoea and NSGI and social class variations in selected categories*

	<i>All registrations</i>			<i>Separated/divorced</i>			<i>Unemployed</i>		
<i>Social class</i>	<i>No.</i>	<i>% with gonorrhoea</i>	<i>% with NSGI</i>	<i>No.</i>	<i>% with gonorrhoea</i>	<i>% with NSGI</i>	<i>No.</i>	<i>% with gonorrhoea</i>	<i>% with NSGI</i>
I, II, and III(N)	529	33.1	15.7	91	46.2	12.1	58	36.2	10.3
III(M), IV, and V	763	46.1	11.0	171	50.3	5.8	108	61.1	3.7
Unclassified	380			25			20		
All classes	1672	37.6	13.3	287	48.4	8.4	186	52.7	5.9

Table 7 *Employment status and diagnosis*

Employment status	Diagnosis										Total no. of registrations	% of total registrations
	Syphilis		Gonorrhoea		NSGI		Other condition(s)		Nil			
	No.	%	No.	%	No.	%	No.	%	No.	%		
Employed	5	0.6	272	34.5	117	14.8	103	13.1	316	40.1	789	47.2
Unemployed	7	3.8	98	52.7	11	5.9	20	10.8	57	30.6	186	11.1
Housewives	12	2.7	214	48.0	55	12.3	29	6.5	147	33.0	446	26.7
Students	0	0	24	13.8	36	20.7	20	11.5	97	55.7	174	10.4
Schoolgirls	0	0	20	26.0	4	5.2	9	11.7	44	57.1	77	4.6
Total	24		628		223		181		661		1672	100.0

The incidence of gonorrhoea and of syphilis was highest among those describing themselves as unemployed. The distribution of diagnoses in students resembled that of social classes II and III rather than that of social classes IV and V; this contrasts with the general pattern in the corresponding age group.

MEDICAL HISTORY AND DIAGNOSIS

Patients previously examined in the clinic, or at other clinics, were classified as 'repeaters'; others were classified as 'new patients'. Patterns of diagnosis in these categories are shown in Table 8.

Discussion

Little systematic evidence is available on the social distribution of sexually transmitted diseases in the UK. The results of this study suggest that women in certain social categories may be particularly vulnerable to sexually transmitted infections. They also illustrate definite differences in the social distribution of different infections among clinic patients.

MARRIAGE BREAKDOWN

The number of cases presenting in separated or divorced women amounted to nearly three-quarters (74.2%) of the number presenting in married patients living with their husbands. Separated or divorced women accounted for 17.2% of total registrations; this compares with 9% of male

registrations accounted for by patients in this category during a parallel survey of cases examined in the Hull men's clinic (Heywood and Bacon, 1975). Interestingly, the results of other clinic surveys suggest that separated and divorced patients may, in general, account for a higher proportion of female than of male clinic cases (Pemberton *et al.*, 1972; Satin and Mills, 1978).

UNEMPLOYMENT

'Unemployed' patients accounted for 11.1% of cases examined and for 16.1% of total diagnoses of syphilis and gonorrhoea. High levels of unemployment among female patients with infectious syphilis and gonorrhoea have been recorded elsewhere (Satin and Mills, 1978). The average monthly rate of female unemployment (as calculated by the Department of Employment) amounted to between 1% and 2% in the north Humberside area in each year of the survey period, although the rate among teenagers may have been somewhat higher (Department of Employment, 1970-73).

SOCIAL CLASS PATTERNS OF DIAGNOSIS

Social class differences in patterns of diagnosis among clinic patients have been recorded previously. In particular, gonorrhoea occurs more frequently, and NSGI less frequently, in lower social classes than in higher social classes (Pemberton *et al.*, 1972; Heywood and Bacon, 1975). This study confirms these findings, although the class differences were

Table 8 *Medical history and diagnosis*

Medical history	Diagnosis										Total no. of registrations	% of total registrations
	Syphilis		Gonorrhoea		NSGI		Other condition(s)		Nil			
	No.	%	No.	%	No.	%	No.	%	No.	%		
New patients	12	0.9	474	36.5	177	13.6	147	11.3	526	40.5	1300	77.8
Repeaters	12	3.2	154	41.4	46	12.4	34	9.1	135	36.3	372	22.2
Total	24		628		223		181		661		1672	100.0

less marked than the corresponding differences among patients examined in the Hull men's clinic (Heywood and Bacon, 1975).

An approximately inverse relationship between the incidence of gonorrhoea and of NSGI was evident not only between social classes but between other demographic and social categories of patients. These distributions cannot be adequately accounted for in terms of the social class distribution of patients between these categories. Unemployment and marriage breakdown were associated with a higher incidence of gonorrhoea at each social level. Similarly, diagnoses of NSGI were less frequent among separated/divorced and unemployed patients than among others in the same social class.

GONORRHOEA AND NSGI

While the social distribution of gonorrhoea can be interpreted in terms of the extent of promiscuity and the social character of consorts, variations in the incidence of NSGI seem to require a quite different explanation. In this study NSGI was diagnosed mainly by inference in contacts of men with non-specific urethritis (NSU). 'Psychological factors' have been referred to in the symptomatology of NSU in male patients (Evans, 1978). Whatever the nature of the variables predisposing to this condition, they

appear to be predominantly associated with professional and white-collar occupations and inversely related to some of the variables predisposing to infection with gonorrhoea:

This paper is based on work undertaken with the aid of a research grant from the Social Science Research Council. The author gratefully acknowledges the help and advice given by Dr C. P. Heywood of the Hull Royal Infirmary.

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Sexually transmitted diseases in Ontario

R. D. CATTERALL

From James Pringle House, The Middlesex Hospital, London

Introduction

Canada is a federal union of 10 provinces and two territories. It is an independent community within the British Commonwealth of Nations. The constitution still rests on the British North America Act of 1867 and, despite repeated attempts to agree to a new constitution, little progress has been made. The population of Canada in 1977 was 22 191 000, of whom eight million were of British origin, five and half million of French origin, and one million of German origin. An increasing number of immigrants from Asia and the Pacific is now being reported.

The Federal Government and the Parliament in Ottawa control foreign policy, major economic affairs, and general home policies. The provincial governments have full sovereignty in all local matters. Responsibility for the provision of health care in Canada is vested in the provincial governments.

In the 1950s and 1960s the Federal Government gave direction and help to the provincial governments to establish health insurance schemes. There were differences of detail in each province, but the general guidelines were: (a) that all Canadian citizens should be eligible for benefits without any exception; (b) that care should be easily accessible to all; and (c) that schemes should be run on a non-profit-making basis and should be transferable between provinces.

At first the Federal Government shared the costs with the provinces but it has recently transferred the major financial responsibility to the provinces. It is estimated that the schemes cost about 7.1% of the gross national product.

Ontario Health Insurance Plan

Ontario is one of the most densely populated provinces situated close to the great lakes. It is an industrialised province with a population of about 7 700 000 people. Toronto is the principal town and

major administrative centre, with highly developed industries, important banking and publishing interests, and a large port.

Until 1969 health care covered only hospital expenses. In 1969 the Ontario Health Services Plan started to cover outpatient service costs. The current Ontario Health Insurance Plan (OHIP) now provides cover for doctors' fees, laboratory and radiological investigations, occupational therapy, chiropody, optometry, chiropractice, osteopathy (up to \$125 a year), ambulance services, and some home nursing.

The scheme does not cover the cost of drugs, dental care, spectacles, false teeth, hearing aids, nor cosmetic surgery. There is a drug benefit scheme for old-age pensioners and those receiving social security. About three-quarters of those who are not eligible for free drugs belong to private insurance schemes, which are often part of fringe benefits negotiated with employers.

The subscription to the OHIP is \$19 a month or \$38 for family cover. People over 65 are exempt and the scheme is optional for the self-employed and those in small firms.

DOCTORS' FEES

There are 16 500 licensed doctors in Ontario. They are paid on an item-of-service basis. When a patient consults a doctor or goes to a medical institution a form is filled in at each visit. This includes the patient's name, address, date of birth, the OHIP registration number, and the doctor's name, number, and address. The date of examination and the diagnosis, together with the scheduled fee, is recorded, as is the type of service provided—such as office or home or hospital consultation.

It is estimated that about 12 000 of the 16 500 licensed doctors regularly submit claims to the OHIP. Some doctors opt out of the scheme and negotiate their fees directly with their patients. The patients can claim back the standard fee for the service on the OHIP schedule but have to pay the difference between the two fee scales.

Over six million claims are dealt with each month and there is a clerical staff of over 2000 employees. The administrative costs are estimated to be between 5% and 6% of the one billion dollars paid out each year in claims. Everyone in the scheme must have an

Address for reprints: Dr R. D. Catterall, James Pringle House, Middlesex Hospital, London W1A 8AA

Received for publication 24 January 1979

OHIP number which is given only to the heads of households. When they are 21 years of age, or when they marry young, people are given an independent number but otherwise they must use their father's number.

Fee schedules are negotiated annually with the Ontario Ministry of Health by the Ontario Medical Association. The fee for an office visit to a family physician is at present \$8.75 and for a specialist \$30-60 under OHIP. In the private sector an office visit costs about \$12 for a family physician and significantly more for a specialist consultation.

DOCTORS' CLAIMS AND INCOMES

Claims submitted by doctors to the OHIP are all carefully monitored by the Professional Services Monitoring Board of the Health Insurance Division of the Ontario Ministry of Health, using a computer which checks the accuracy of the claims and arranges payments. The computer also produces a picture of each doctor's professional habits and compares them with those of his colleagues in similar specialties. It can note any divergence from the practices of his colleagues and records the use of laboratory and radiological services.

Medical incomes in Canada are high and the average earnings of family doctors in 1977 were about \$46 000, or £24 000 before tax. Incomes of specialists are substantially higher. The paperwork in submitting claims for fees to the OHIP is tedious and onerous, occupying about one hour a day for each doctor. The fee-for-service system is open to obvious abuse by both doctors and patients. All OHIP employees are legally bound not to divulge medical information to a third party but computerised information makes real confidentiality difficult, and there appears to be greater access to medical information than would be allowed in the United Kingdom.

Sexually transmitted diseases

Patients who suspect that they may have a sexually transmitted disease (STD) usually go to a family or private physician and not to a specialised clinic. It is estimated that between 70 and 80% of these patients either attend their own physician or go to see another physician in his office. Only about one patient in five attends a Department of Health venereal diseases clinic. The fee for consultation is \$8.75 and can be claimed from the OHIP. Laboratory fees are about \$4 but darkground microscopy is \$9. Serological tests for syphilis are free and are carried out by the public health laboratories.

STD CLINICS

The STD clinics are organised by the senior medical

consultant and principal programme adviser on STD to the Ontario Ministry of Health. The Venereal Diseases Prevention Act of 1918 states that all hospitals have the right to establish STD clinics provided the medical officer of health agrees to the arrangements. Contacts of known patients who are suspected of being infected can be compelled by law to be examined and treated. In the clinics all examinations, investigations, and treatments are free. The patients are not asked to prove that they are covered by the OHIP scheme.

There are 32 clinics for patients with STD in Ontario serving a population of 7.7 million people. About 80% of the population of Ontario live in urban areas and there are 12 clinics in Toronto, one in Ottawa, one each in London, Hamilton, and Kingston, and others in smaller centres. Of the 32 clinics, only 16 are in hospitals, the remainder being situated in health units away from the hospitals. All the clinics are open only part-time each day.

FINANCE

The total cost of the clinics is paid by the Department of Health. The clinics are under the general direction of the local medical officer of health and the doctors who work in them all have major interests in other fields, such as dermatology, gynaecology, urology, or family medicine. There is no such person as a pure venereologist. Doctors are paid a fee of \$8.75 for each patient seen in the clinics, whether they are new patients or those attending for follow-up examinations.

The estimated cost of each patient-visit is about \$32 and, in addition to the funds from the Department of Health, the hospital or health unit contributes an extra sum of \$8 per patient towards clinic expenses. The clinical nurses are paid by the hospital or health unit and the public health nurses, who do the contact-tracing, by the medical officer of health. In some clinics there are laboratory technicians who are paid \$3.84 for every stained microscope slide examined.

CLINIC ATTENDANCES

The total number of attendances of all patients at the 32 clinics was 165 000 in 1977. It is recommended that the ideal patient flow in clinics should be of the order of seven to eight patients per hour for each physician.

TEACHING

Medical students have some lectures on STD at most medical schools but no clinical experience with patients. In some schools the subject is only mentioned in lectures on gynaecology and urology, and there is no planned teaching of the subject. Even

a very modern medical school like McMaster University Medical School in Hamilton has no planned course of teaching about the diseases. Thus the students' only opportunity to learn about the subject is if they choose it as an elective subject, but unfortunately few do this. Opportunities for postgraduate study are few and, where they do exist, take the form of lectures.

NOTIFICATIONS

All family doctors, specialists, and private physicians are required by law to notify all cases of STD to the local medical officer of health. Records of all cases are then forwarded to the director of public health services in Toronto. As is usual with such a system there is considerable under-reporting. For example, in 1977 the total number of reported cases of gonorrhoea was about 20 000 and, of syphilis, 2200. It is estimated that the actual true totals should be nearer 60 000 cases of gonorrhoea and 2500-3000 cases of syphilis. The incidence of all the other sexually transmitted diseases is unknown.

CONTROL PROGRAMMES

Ontario, Quebec, and British Columbia are the only three provinces which have developing programmes for the control of sexually transmitted diseases, and it is reasonable to conclude that the prevalence and economic importance of the diseases are completely unknown in the other provinces and territories.

Discussion

The methods developed in Ontario for the control and prevention of sexually transmitted diseases are closer to the system used in the United States than to the British system. Essentially they are based on a series of programmes which are initiated and controlled by the Public Health Service through the senior medical consultant and principal programme adviser on STD to the Ontario Ministry of Health. As in the United States about 80% of patients are seen by their family physicians or by private doctors and only about 20% attend the clinics. Unfortunately, very few family or private physicians have been taught anything about the management of patients with STD at their medical schools, and postgraduate teaching in the subject is still rudimentary. As a result standards of diagnosis and treatment are very variable and contact-tracing is often omitted.

The clinics themselves are frequently not part of hospitals but are relatively isolated units, and the doctors working in the clinics are not fully committed to the specialty and usually have another major interest such as family medicine, dermatology,

urology, or gynaecology. There is no equivalent of the British consultant physician, who has had postgraduate training in general medicine and three to four years of specialist training in STD at a large clinic in a teaching hospital.

Some of the clinics in Ontario treat only patients with statutory venereal diseases. This makes the work limited and unattractive and leads to difficulties in recruiting high-quality doctors. A broader concept of the subject—including a wide variety of diseases of the genitourinary systems—could well improve recruitment and even persuade some doctors to make a career in the subject.

As is the case all over the world the standards of the premises are very variable. The best clinics are well equipped and are housed in reasonable departments, in some cases in university hospitals. Other clinics are overcrowded and in unattractive surroundings, often outside the main hospital, and are unlikely to attract either patients or well-trained physicians and nurses. Because of the shortage of doctors, nurse assistants are widely used and they carry out many of the duties which have traditionally been the responsibility of doctors. The public health nurses who undertake the contact-tracing have no formal training in the subject at present but they perform their duties with enthusiasm and considerable success.

Conclusions

The Ontario Ministry of Health recognises that the sexually transmitted diseases constitute an important problem in the province. Through their senior medical consultant in STD they are taking active steps to develop a service for patients and plans to try to control the incidence of the diseases.

University hospitals do not provide adequate undergraduate teaching about sexually transmitted diseases and many of them do not have a clinic. As a result medical students rarely have opportunities for clinical teaching on patients. It is something of a paradox that most infected patients are treated by family and private physicians who have had no training in the subject. Furthermore, if medical students do not receive adequate teaching—including clinical experience with patients—recruitment to the specialty will remain poor and the best students will not consider the specialty as a career.

There are great opportunities for the development of a high-quality service in Ontario. The essential step is to establish the subject in all the university hospitals and to ensure that medical students receive both theoretical teaching and practical experience with patients. One of the initial difficulties will be to

find suitable people to do the teaching and this can only be achieved in the long run by establishing university hospital clinics and providing a career structure in the specialty. Most other improvements will follow such as attractive clinics, higher standards of clinical practice, better teaching and research, and the improved training of nurses, social workers, and other ancillary workers.

Meanwhile, during the transition period—before the benefits of this policy become apparent—a major attempt could be made to educate practising physicians and to keep them up to date with recent developments in the subject. This could take the form of postgraduate seminars, workshops, incentives, the circulation of recommended diagnostic procedures, and treatment schedules, and

the formation of a group of public health workers who could be available to physicians to help with the management of their patients and the tracing of contacts. Experience in the United States has shown that this approach has its limitations. Education and clinical experience during the formative years are more effective than attempts to change unsatisfactory habits acquired through lack of guidance and instruction.

Ultimately the establishment of a high-quality service for the control of sexually transmitted diseases could have important consequences for the health of the community. It could be shown to be very cost-effective in preventing the complications of the diseases, which often result in repeated periods of hospitalisation and prolonged ill-health.

Australian conference on STD

Report of the first national conference on STD held in Perth, Western Australia, 11-13 August 1978

Australia's first national conference on STD was held in Perth in the state of Western Australia from 11 to 13 August 1978. Four hundred participants were present, of which 100 were doctors, 40 of them coming from other Australian states. The remainder were mainly nurses, but wide interest was shown by laboratory technicians, social workers, medical students, and school teachers. This cross-section of medical and para-medical health workers gave the conference a special quality of reality.

The keynote paper reviewed the STD situation from a worldwide point of view, paying particular attention to the size of the problem and its social determinants. There were references to the situation in Australia. Apart from Western Australia, states have been slow in coming to grips with the problem. It was clear that more than just treatment of individuals with symptoms was needed. The cost of complications and preventable disability from STDs was unnecessarily high in Australia. An efficient and effective programme of STD control could be among the most cost-effective investments Australian states could make. To prove this, it was suggested that all such endeavours should have built in evaluation studies.

Demographic reports from Australia came from Dr W. A. Langsford, of the Federal Health Department, and those concerning Western Australia from Dr W. A. Newnham. These reports concentrated almost entirely on syphilis and gonorrhoea.

Reports from laboratory workers were a feature of the conference. Dr V. Blackman sketched the history of syphilis serology and described the reagin tests. Associate Professor R. Dawkins reported on the modern approach concerning the more specific test; the treponemal haemagglutination (TPHA) test was favoured. The fluorescent treponemal antibody (FTA-ABS) test was most usefully deployed as the monospecific tests—the FTA-IgG and FTA-IgM. Other laboratory papers covered culture of *Chlamydia trachomatis* (Dr P. Phillips), herpes virus studies (Mr G. Hartnett), and the laboratory diagnosis of

granuloma inguinale (Dr A. Henderson), a condition which is still regularly seen in Western Australia.

Two papers were read concerning treatment. Professor S. Goodwin discussed the philosophy of antibiotic use in STDs, while Dr L. Laden pointed out that Australia is particularly vulnerable to invasion by β -lactamase-producing gonococci. From WHO reports Australia has the third highest number of isolates after the USA and the UK. Beta-lactamase-producing gonococci have been regularly imported by Australians touring South-East Asia and the Western Pacific over the last two years. Spread to home contacts has apparently not gone beyond the first generation. Considerable anxiety about the possibility of the spread of these costly organisms came from the floor.

Clinical observations from general practitioners were reported by Dr F. McConnel and Dr J. Leavesley. It emerged from the ensuing discussion that two fifth-year medical students had undertaken a questionnaire to survey the amount of STD treated in the Perth metropolitan area. This showed that the bulk of STD work was still being dealt with in the private sector. Reports from hospital clinics emphasised the current epidemic of genital herpes. Dr M. Gollow reported the finding of herpes virus in the urethra of six men without a history or clinical evidence of the disease. Dr G. Kelly produced epidemiological evidence to show that, while syphilis and gonorrhoea were apparently being brought under control, this was certainly not the case with the five viral STDs—least of all genital herpes.

Bridging the gap between medical and social aspects, Mr Frank Hillman reported on the methods and outcome of contact-tracing in the Perth Special Clinic. Four thousand men with syphilis or gonorrhoea were persuaded to name 1500 contacts over a two-year period. Emphasis was placed on the need for general practitioners to avail themselves of this service. Also in this category was a paper entitled 'New Approaches to Health Education about the STDs' read by Mr Colm O'Doherty of the Perth Health Education Department. Some

of the audiovisual aids in daily use were demonstrated in a caravan. Dr F. Quadros and Mr Collard dealt objectively and sensitively with the size and nature of the STD problem among Aboriginal people.

The two socially orientated papers had attractive titles. The first, 'My Doctor Never Tells Me Anything', was the report of a registered nurse (Miss J. Watson) and an obstetrician (Professor P. Giles). The second paper, 'Grandmother's Risks and my Daughter's Hazards' was read by Dr Anne Troup, a general practitioner with STD experience, and by Mr R. Smith, a schoolmaster at a major high school. All four speakers were both informative and provocative. Discussion—largely promoted by non-medical delegates—was lively.

The final subject on the agenda, 'Is There a Place for STD Education in the Medical Students' Curriculum?' had two main speakers. Dr G. Hart succinctly put forward the case of need, underlining the fact that although STDs had been increasing year by year for 20 years or more, medical schools in many parts of the western world had continuously neglected them. Professor D. Allbrook, associate dean in the Perth faculty, circulated a review showing how little was done about the subject in the medical schools of Australia. Although attention in laboratory courses, and occasionally in behavioural sciences and social medicine courses, was evident, students' contact with patients with STD was everywhere virtually non-existent. Contributions to the discussion by medical students aired criticisms of the local faculty's failure in this area. Other contributors criticised the faculty's lack of appreciation of what changing attitudes and behaviour had meant to the day-to-day practice of medicine. All the contributors were clearly bent on action.

Nobody left this conference without being aware that, although Australia's entry into the field of STD control is somewhat belated, it is certainly spirited. Her growing enlightenment, led so ably by Dr W. A. Newnham and his staff in Western Australia, will certainly be welcomed by her neighbours.

R. S. Morton

Correspondence

TO THE EDITOR, *British Journal of Venereal Diseases*

Effect of allergy on response to treatment of nongonococcal urethritis

Sir,

Nongonococcal urethritis (NGU) is almost certainly not a single disease entity. Although it is widely accepted that urethral infection, notably by *Chlamydia trachomatis*, is a major factor in the pathogenesis (Holmes *et al.*, 1975), there are many cases—particularly of recurrent NGU—where the aetiology is obscure and the results of therapy variable and irregular. Previous searches for an immune mechanism (Csonka *et al.*, 1974) have been inconclusive, but I describe here an apparent association between a poor clinical response to antimicrobial treatment and a history of hypersensitivity or allergic reactions.

times daily for two weeks. They were then divided into those who showed symptomatic improvement associated with a reduction in the number of urethral pus cells to less than 10 per high-power field ('responders'), and those who did not ('non-responders').

Of the 198 patients diagnosed as having NGU only 51 (26%) could be included in the study for various reasons, which included failure to attend for follow up. Twenty-seven of these were new cases of NGU and 24 had a second or subsequent attack. A history suggestive of allergic reactions was given by 19 (35%) of the patients; and such patients, whether they had first attacks of NGU or recurrences, were less likely to respond to the initial course of antibiotics than were normal patients. This difference is most noticeable among patients with recurrences (Table).

Because of the small sample size both new cases and recurrences were considered

References

- Csonka, G. W., *et al.* (1974). Raised levels of an unknown betaglobulin in the serum of patients with non-specific urethritis and Reiter's disease. *British Journal of Venereal Diseases*, 50, 17-21.
Holmes, K. K., *et al.* (1975). Etiology of nongonococcal urethritis. *New England Journal of Medicine*, 292 (23), 1199-1205.
Weston, T. E. T. (1965). An allergic basis for non-specific urethritis. *British Journal of Venereal Diseases*, 41, 107-116.

TO THE EDITOR, *British Journal of Venereal Diseases*

Management of non-specific urethritis in men

Sir,

In their lengthy and detailed assessment of various tetracycline treatments of non-specific urethritis, Arya *et al.* (1978) evaluated the effect of empirical treatment of contacts in a sample of 112 patients from the total of 562 patients who were adequately followed up. The overall re-treatment rate in the whole trial group was 27% whereas the retreatment rate in the sample was 46%. This difference is statistically significant (χ^2 18.662 with Yates's correction; $P < 0.001$). In other words, the sample (selected by contact attendance) was not representative of the trial group as a whole, and the conclusion that 'treatment of sexual contacts before resumption of sexual intercourse significantly reduced the retreatment rate' is not valid. In fact, the 'contact treated before further intercourse' group had much the same retreatment rate (25%) as was found in the trial as a whole.

Why should the retreatment rate be so high in the sample with a contact attendance? May I suggest that this was biased by treatment failure among those in whom it was delayed? Unfortunately no figures are given for this important interval.

Yours faithfully,

Brian Evans

West London Hospital,
London W6 7DQ

Reference

- Arya, O. P., Alergant, C. D., Annels, E. H., Carey, P. B., Ghosh, A. K., and Goddard, A. D. (1978). Management of non-specific urethritis in men: Evaluation of six treatment regimens and effect of other factors including alcohol and sexual intercourse. *British Journal of Venereal Diseases*, 54, 414-421.

Table Results of treatment of 51 patients with NGU

Diagnosis of NGU	History of allergy	No. of patients treated	No. failing to respond
First attack	Present	9	3
	Absent	18	3
Second or subsequent attack	Present	10	7
	Absent	14	1

For this pilot study all new and recurrent cases of NGU in men attending the Department of Genitourinary Medicine at University College Hospital over a period of two months were assessed. The diagnostic criteria were: complaint of dysuria, discharge or urethral discomfort, visible discharge or meatal inflammation, and the presence of more than 10 polymorphonuclear neutrophils per microscopic field using $\times 100$ objective and $\times 10$ eye piece of the urethral smear of symptomatic patients from whom no gonococci were isolated and who had no evidence of *Trichomonas vaginalis* or other recognisable genital infection. All these patients were asked if they had any history of allergic diseases including asthma, eczema, allergic rhinitis, or skin sensitivity to substances other than drugs.

All patients in the study group were treated with oxytetracycline 250 mg four

together for statistical purposes. The difference between the response of normal and allergic patients is significant to $P < 0.01$. These results suggest that the possibility of an 'allergic' basis for some cases of NGU should be reassessed (Weston, 1965). One possible mechanism is that in most cases NGU is initiated by an exogenous infection, for example, with *C. trachomatis*, which then sensitises the urethra of a susceptible individual to a number of probably non-infective stimuli.

My thanks to Dr J. D. Oriel for his encouragement and access to his patients.

Yours faithfully,

J. H. Yoxall

James Pringle House,
Middlesex Hospital,
London W1N 8AA

Book reviews

Immunobiology of *Neisseria gonorrhoeae*. Edited by G. F. Brooks, E. C. Gotschlich, K. K. Holmes, W. D. Sawyer, and F. E. Young, 1978. Pp. 422. American Society for Microbiology, Washington DC (\$15).

The last 10 years have seen an enormous research effort aimed at understanding the biology of *Neisseria gonorrhoeae* and how the gonococcus interacts with its unique natural host, man. In January 1978 a conference was held in San Francisco to review our current understanding of this organism and to assess future prospects for the control of gonorrhoea.

This book contains the proceedings of the San Francisco meeting and comprises some 70 papers by leading investigators in the field of gonococcal research. The contents are grouped under six main headings.

The first section contains papers on the physiology, genetics, and antimicrobial susceptibility of the gonococcus and on gonococcal β -lactamases. The second section reviews the surface structures of the gonococcus with respect to their role as cellular antigens. The possibility that gonococcal pili protein may be a useful candidate for a vaccine is discussed in some detail by Brinton and his colleagues. The next two sections are concerned with 'Pathogenicity and Immunity' and 'Immunity and Vaccine' and deal with the host-parasite relationship *in vivo* and animal models of gonococcal infection.

The fifth section, 'Current Concepts and Future Strategies', is a mixture of topics and includes papers on the various

immunological tests which are presently being evaluated for the serodiagnosis of gonorrhoea. The final section, by John Robbins, considers whether a vaccine for gonorrhoea is a realistic possibility.

At the end of each section is a discussion summary which contains comments and ideas from the participants at the conference. Although some papers contain little descriptive methodology, the reader is directed to the list of references at the end of each paper.

The editors are to be congratulated for the rapid publication of this book, which serves as an excellent review of the current status of gonococcal research and a rich source of references to original papers on *Neisseria gonorrhoeae*. It will be of great value to both clinical and non-medical scientists interested in gonococcal research and is to be recommended to all workers interested in the host-parasite relationship in infectious diseases.

P. R. Lambden

Manual of Dermatologic Therapeutics With Essentials of Diagnosis. Kenneth A. Arndt, 1978. Second edition, pp. 337. Little, Brown and Company (Inc.), Massachusetts (\$10.95).

This paperback deals with disorders of the skin seen in ambulatory patients. It is divided into three parts. The first part defines the skin disorders and discusses their aetiology, diagnosis, and treatment. It includes 17 colour photographs of high

quality. The second part outlines common outpatient operative procedures and diagnostic and therapeutic techniques. The third part discusses the principles of treatment, provides an extensive formulary of topical and systemic medications, and gives detailed accounts of the composition of drugs used as well as their possible modes of action. The format is excellent and the material set out in a concise and systematised manner, enabling the reader to pick up the essentials almost at a glance.

The first part of 219 pages contains a chapter of 15 pages devoted to the sexually transmitted diseases (STDs). In fact, more space is allotted to STDs, as conditions such as candidosis, genital herpes, and genital warts are described in other parts of this section.

The information on STDs is generally accurate and to the point, but there are a few errors and omissions. The urethral discharge of nongonococcal urethritis is described as white or clear and that of gonorrhoea as mucopurulent. Although disseminated gonococcal infection and pelvic inflammatory disease are dealt with, epididymitis and Reiter's disease are omitted. No mention is made of idoxuridine in the treatment of the genital lesions of primary herpes.

These are minor criticisms of an otherwise admirable book. More colour photographs would have enhanced its value—but likewise its price. It would be a useful addition to the departmental library and can be highly recommended to medical students and junior medical staff.

C. S. Ratnatunga

Notice

The Specialist Advisory Committee of the Joint Committee for Higher Medical Training in association with the Council of the MSSVD have been considering the promotion prospects for senior registrars in the UK. Because of the expansion of the senior registrar grade prospects for promotion are now less for those without a higher qualification. Excellent career prospects remain for bright young men and women entering the specialty at SHO and registrar grades provided they obtain an appropriate higher qualification.

Abstracts

These selected abstracts and titles from the world literature are arranged in the following sections:

Syphilis and other treponematoses

(Clinical and therapy; serology and biological false-positive phenomenon; pathology and experimental)

Gonorrhoea

(Clinical; microbiology; therapy)

Non-specific genital infection

Reiter's disease

Trichomoniasis

Candidosis

Genital herpes

Other sexually transmitted diseases

Public health and social aspects

Miscellaneous

Syphilis and other treponematoses (Clinical and therapy)

Neurosyphilis today

L. LUXON, A. J. LEES, AND R. J. GREENWOOD (1979). *Lancet*, 1, 90-93

In 17 cases of neurosyphilis, referred from nine other hospitals to the National Hospital, Queen's Square, London, the clinical presentations were diverse, and some are described in detail. Pupillary abnormalities were the commonest sign in the series (nine of 17), including three cases of Argyll-Robertson pupils. External ophthalmoplegias, optic neuritis, optic atrophy, choroidoretinitis, and papilloedema were also described. Classical tabes dorsalis, tabo-paresis, eighth nerve lesions, and complex cerebrospinal involvement were documented, but many cases had more than one of these features. The authors point out that the clinical features of these patients did not differ from those described in the pre-antibiotic era. Diagnosis was confirmed by positive results to specific treponemal tests on specimens of CSF. Results of reagin tests on CSF samples suggest that these may be a less sensitive detector of neurosyphilis than the TPHA and FTA. Ten of the patients had previously received antibiotic treatment for other illnesses.

The response to penicillin, where documented, seemed on the whole better in ocular or otological disease than in spinal or cerebral disease. There was no effect on the Argyll-Robertson pupils. It would, however, be helpful to have more information on the dosage used and the

stage of infection when treated, particularly in the ocular or otological cases. This is a very useful paper for young physicians of the antibiotic era who have seen little classical neurosyphilis. It is a pity it is not presented in greater detail.

David Goldmeier

Bell's palsy and secondary syphilis: CSF spirochaetes detected by immunofluorescence

L. E. DAVIS AND S. SPERRY (1978). *Annals of Neurology*, 4, 378-380

The history of a 30-year-old man, who initially presented with a mild headache, right-sided facial weakness, and a maculopapular rash on the trunk and extremities, is reported from Albuquerque, New Mexico. Bell's palsy was diagnosed, and he was given prednisolone 60 mg/day for two weeks. The rash disappeared but the headaches continued and a mild tinnitus developed. There was no history of a primary syphilitic lesion. Six weeks later the patient was re-examined. There was a right-sided peripheral facial paralysis and a patchy alopecia. Other cranial nerves were normal apart from a bilateral high-tone deafness.

Examination of the CSF at this time showed a raised pressure, 125 white cells/mm³ (80% lymphocytes), and a raised protein (1.060 g/l). The CSF-VDRL test was reactive undiluted and the CSF-FTA-ABS test showed 1+ fluorescence. Serum tests gave RPR positive results (1/128) and the FTA-ABS test strongly positive results. The patient was treated for three weeks with penicillin (the dosage and preparation are not recorded). One week later movement had returned to the right half of

the face, and the headaches and tinnitus had gone. The CSF-VDRL test became non-reactive, and the CSF-FTA-ABS test was borderline reactive. The CSF biochemical results became normal.

A centrifuged specimen of the CSF taken before treatment showed 15 treponeme-like organisms in the deposit. These gave specific fluorescence when stained indirectly with known positive serum. Adequate controls were used. No treponeme-like organisms were seen in the post-treatment specimen similarly examined.

This paper re-emphasises the value of a close working relationship between otorhinolaryngologists and venereologists.

G. D. Morrison

Syphilis (pathology and experimental)

Influence of oxygen tension, sulphhydryl compounds and serum on the motility and virulence of *Treponema pallidum* (Nichols strain) in a cell-free system

S. J. NORRIS, J. N. MILLER, J. A. SYKES, AND T. J. FITZGERALD (1978). *Infection and Immunity*, 22, 689-697

The survival of virulent *Treponema pallidum* in Eagle's MEM medium with various additives was studied under aerobic and anaerobic conditions and in the presence of 3% oxygen. The addition of 50% inactivated normal rabbit serum prolonged motility under anaerobic conditions and in the presence of 3% O₂ but not in aerobic cultures. The addition of L-cysteine did not help survival but

glutathione had some effect. Dithiothreitol (DTT) prolonged survival for up to 180 hours in an environment of 3% O₂ but had no effect in the anaerobic system; its effect was decreased in the presence of L-cysteine and glutathione. The optimum concentration of DTT was found to be 1.0 mmol. In further experiments this concentration was shown to promote retention of virulence of the organism. The basal medium plus serum and DTT was found suitable for the growth of cultures of normal rabbit testis cells in the presence of 3% O₂ but not under anaerobic conditions.

The mode of action of sulphhydryl compounds and DTT is discussed. The results obtained suggest that *T. pallidum* is a microaerophilic organism rather than a true anaerobe.

A. E. Wilkinson

(Reprinted from *Abstracts on Hygiene* by permission of the Editor.)

Capacity of virulent *Treponema pallidum* (Nichols) for deoxyribonucleic acid synthesis

J. B. BASEMAN, J. C. NICHOLS, AND S. MOGERLEY (1979). *Infection and Immunity*, 23, 392-437

Gonorrhoea (Microbiology)

Serology of *Neisseria gonorrhoeae*. Demonstration of strain-specific antigens by immunoelectrophoresis, immunofluorescence and co-agglutination techniques

D. DANIELSSON AND E. SANDSTRÖM (1979). *Acta Pathologica et Microbiologica Scandinavica*, Sect B, 87, 55-64

Four strains of gonococci isolated from two patients with complicated and two with uncomplicated infections were studied. The first two strains had the same, and the last two different, auxotypes. Antisera were raised against these strains in rabbits and tested against sonicates of the various strains by crossed immunoelectrophoresis, crossed line and rocket electrophoresis, and by indirect immunofluorescence and co-agglutination tests with intact organisms. Strain-specific antigens were demonstrated; these resisted heating at 100°C but not at 120°C and were probably constituents of the outer membranes. It was noted that in the co-agglutination test staphylococci

coated with unabsorbed or absorbed antibodies gave weak or negative results with untreated gonococci of T3/T4 colonial types but strong reactions with T2 organisms. After heating T3/T4 gonococci at 100°C for an hour they reacted as strongly as T2 organisms.

A. E. Wilkinson

(Reprinted from *Abstracts on Hygiene* by permission of the Editor.)

Auxotypes and antibiotic susceptibility patterns of *Neisseria gonorrhoeae* from disseminated and local infections

S. E. THOMPSON, G. REYNOLDS, H. B. SHORT, C. THORNSBERRY, J. W. BIDDLE, N. F. JACOBS, M. F. REIN, A. A. ZEIDI, F. E. YOUNG, AND J. A. SHULMAN (1978). *Sexually Transmitted Diseases*, 5, 127-131

The arginine-hypoxanthine-uracil auxotype has been linked with the propensity of gonococci to cause disseminated infections. Gonococci recovered from 25 patients with disseminated gonococcal infections were compared with gonococci recovered from matched controls, patients with uncomplicated gonorrhoea selected during the same month. Minimum inhibitory concentrations of penicillin, tetracycline, erythromycin, and ampicillin, and the nutritional requirements (auxotypes) for proline alone, arginine alone, arginine, hypoxanthine and uracil together, serine alone and cysteine-cystine (wild type) were analysed by discriminant analysis. Significant susceptibility to penicillin characterised strains causing disseminated infections, and a proline requirement was the most common auxotype (48%) among strains isolated in Atlanta. Together the minimum inhibitory concentration of penicillin and the proline auxotype best separated the strains causing disseminated gonococcal infections from those causing uncomplicated gonorrhoea. The arginine-hypoxanthine-uracil auxotype was found in only 24% of strains causing disseminated infections. A trait other than auxotype must determine the capacity of the organisms to disseminate.

Authors' summary

Cell envelope of *Neisseria gonorrhoeae* CS7: peptidoglycan-protein complex

B. H. HEBELER, W. WONG, S. A. MORSE, AND F. E. YOUNG (1979). *Infection and Immunity*, 23, 353-391

Studies on gonococcus infection, XVII. IgA cleaving protease in vaginal washings from women with gonorrhoea

H. M. BLAKE, K. K. HOLMES, AND J. SWANSON (1979). *Journal of Infectious Diseases*, 139, 89-92

Non-specific genital infection

Diagnosis and etiology of non-gonococcal urethritis

S. L. SWARTZ, S. J. KRAUS, K. L. HERRMANN, M. D. STARGEL, W. J. BROWN, AND S. D. ALLEN (1978). *Journal of Infectious Diseases*, 138, 445-455

The observation of more than four polymorphonuclear cells (PMN) per high power field (hpf) in Gram-stained smears of urethral secretions was found to differentiate patients with urethritis from patients without urethritis. A urethral discharge was present in 78% of patients with nongonococcal urethritis (NGU). Dysuria without demonstrable urethral discharge and with fewer than four PMN/hpf did not appear to fit into the NGU spectrum. NGU is now defined to include men who have negative urethral culture results for *Neisseria gonorrhoeae* with a urethral discharge or more than four PMN/hpf in their urethral smears or both. The finding of more than four PMN/hpf in the urethral smears of 22% of asymptomatic sexually active men with more than one sexual partner (polygamous controls) suggests that asymptomatic NGU is not uncommon. *Chlamydia trachomatis* was isolated significantly more frequently from the NGU study group than from the control group ($p < 0.001$). This study adds *Corynebacterium vaginale* (*Haemophilus vaginalis*), group B streptococci, and yeasts to the list of sexually transmitted microorganisms that are not aetiological determinants of NGU.

Authors' summary

Etiologies of postgonococcal urethritis in homosexual and heterosexual men: roles of *Chlamydia trachomatis* and *Ureaplasma urealyticum*

W. R. BOWIE, E. R. ALEXANDER, AND K. K. HOLMES (1978). *Sexually Transmitted Diseases*, 5, 151-154

Before treatment for urethral gonorrhoea, *Chlamydia trachomatis* was isolated from 18% and *Ureaplasma urealyticum* from

37% of 121 men. *C. trachomatis* was recovered from none of 18 homosexual men who had gonorrhoea and from 22 of 95 heterosexual men who had gonorrhoea ($P < 0.05$). After treatment with a penicillin, postgonococcal urethritis occurred significantly more often in heterosexual than in homosexual men ($P < 0.002$). Postgonococcal urethritis developed in all men from whom *C. trachomatis* was isolated. Among men without *U. urealyticum* infection, postgonococcal urethritis was significantly associated with *C. trachomatis* infection ($P < 0.02$). Among men without *C. trachomatis* infection postgonococcal urethritis was less closely associated with *U. urealyticum* infection ($0.1 > P > 0.25$). Postgonococcal urethritis was least frequent among men who had neither *C. trachomatis* nor *U. urealyticum* infection.

Authors' summary

***Chlamydia trachomatis* cervicitis in gynecologic outpatients**

K. T. RIPA, L. SVENSSON, P.-A. MÅRDH, AND L. WESTRÖM (1978). *Obstetrics and Gynecology*, 52, 608-702

Symptoms suggestive of a lower genital tract infection (LGTI) are common complaints in women who consult gynaecologists. Sexually transmitted microorganisms, such as *Chlamydia trachomatis*, are responsible for a substantial proportion of LGTI. This study was performed to establish the frequency of LGTI caused by *C. trachomatis* in women attending a gynaecological outpatient clinic. Of 170 women with LGTI, 32.9% harboured one or more of these organisms: *C. trachomatis* was found in 19.3%, *N. gonorrhoeae* in 4.7%, and *T. vaginalis* in 25.9%. The results of the isolation studies were correlated with clinical signs and symptoms. In women under 25 years of age, chlamydial cervicitis was found significantly more often in users of oral contraceptives than in non-users. In women not taking such drugs, cervical erosion was found more often in chlamydiae-positive than in chlamydiae-negative women. Since clinical examination failed to show any characteristic signs in cases of chlamydial infection, it was not possible to distinguish a chlamydial from a gonococcal infection. This study also reports the successful treatment of 15 women affected by chlamydial cervicitis with doxycycline or trimethoprim-sulphamethoxazole.

Authors' summary

Chlamydial pneumonitis

C. ARTH, B. VON SCHMIDT, M. GROSSMAN, AND J. SCHACHTER (1978). *Journal of Pediatrics*, 93, 447-449

***Chlamydia trachomatis* and pneumonia in infants: report of two cases**

J. A. EMBIL, R. L. OZERE, AND S. W. MACDONALD (1978). *Canadian Medical Association*, 119, 1199-1204

Infection with *Chlamydia trachomatis*: Involvement of multiple anatomic sites in neonates

J. SCHACHTER, M. GROSSMAN, J. HOLT, R. SWEET, AND S. SPECTOR (1979). *Journal of Infectious Diseases*, 139, 232-234

Experimental nasopharyngitis and pneumonia caused by *Chlamydia trachomatis* in infant baboons; histopathologic comparison with a case in a human infant

H. R. HARRISON, E. RUSSELL ALEXANDER, W. T. CHIANG, W. E. GIDDENS JR., J. T. BOYCE, D. BENJAMIN, AND J. L. GALE (1979). *Journal of Infectious Diseases*, 139, 141-146

These four papers on neonatal chlamydial infection may be considered together. Infantile pneumonia associated with *C. trachomatis* was first defined clinically by Beem and Saxon (1977). Characteristically it presents at the age of 4-6 weeks as an afebrile illness with tachypnoea and a paroxysmal staccato cough. Chest x-rays show diffusely distributed interstitial and alveolar infiltrates, and IgG and IgM concentrations are often raised. *C. trachomatis* is usually recovered from nasopharyngeal and tracheal aspirates, and occasionally from other sites, and there are elevated antichlamydial antibody titres as measured by microimmunofluorescence. One half of these infants have conjunctival involvement according to history, examination, or culture. Frommell *et al.* (1977) isolated the organism from lung tissue, but their patient also had a cytomegalovirus infection.

In the infant described by Arth *et al.*, working in San Francisco, the characteristic clinical features were present. Although there was a history of ocular infection a few days after birth (treated with sulfisoxazole), chlamydiae were not isolated from ocular or pharyngeal specimens. However, an open biopsy specimen of lung tissue, which showed near obliteration of involved alveoli with round cells and widespread necrotising bronchiolitis, yielded chlamydiae on

culture. The patient recovered completely after treatment with erythromycin.

Embil *et al.* report two further cases of chlamydial pneumonia in infants from Halifax, Canada. In both, the illness was most severe at age 4 weeks. One had a history of conjunctivitis, and both presented with staccato cough and tachypnoea. Chest x-rays showed hyperexpansion and pulmonary infiltration, and the IgM concentrations were markedly raised. *C. trachomatis* was recovered from nasopharyngeal and throat swabs from both infants, and chlamydial antibody titres were raised in one. One of the babies was treated with gentamicin and ampicillin initially followed by trimethoprim-sulphamethoxazole when the diagnosis became clear, and the other with sulfisoxazole, and they both recovered.

It is of interest that the mothers of all three babies described in these two papers gave a history of vaginal discharge towards the end of pregnancy; *C. trachomatis* was recovered from the cervix of one of these women.

In the third of the papers reviewed, Schachter *et al.*, from San Francisco, describe 12 infants from whom chlamydiae had been recovered. Seven had ocular chlamydial infection. Two of six female infants tested for vaginal infection yielded chlamydiae, and both had synchronous chlamydial conjunctivitis. Chlamydiae were recovered from the respiratory tract of five infants, four from nasopharyngeal or throat swabs or both, and one from lung biopsy material. Two other infants with radiologically confirmed pneumonia yielded chlamydiae only from the rectum. The authors suggest that the timing of recovery indicates that the vagina and conjunctivae are exposed to chlamydiae at birth and that pneumonia and gastrointestinal infection occurs later; rectal specimens may be useful for the diagnosis of chlamydial disease in infants.

Finally, Harrison *et al.* from the University of Washington, Seattle, describe the intratracheal, nasopharyngeal, and oropharyngeal inoculation of three infant baboons with a strain of *C. trachomatis* isolated from a human infant with pneumonia. One animal showed a persistent nasopharyngeal chlamydial infection, radiological evidence of pneumonia, and histological changes almost identical to those seen in human neonatal chlamydial pneumonia. The second baboon maintained nasopharyngeal chlamydiae for 30 days (when it was killed) and had a patchy mild pneumonitis at

necropsy, and the third maintained nasopharyngeal chlamydiae for 49 days. All three animals showed seroconversion. The authors suggest that infant baboons may be a useful animal model for *C. trachomatis* nasopharyngitis and pneumonia.

J. D. Oriel

Beem, M. O. and Saxon, E. M. (1977). Respiratory tract colonisation and a distinctive pneumonia syndrome in infants infected with *Chlamydia trachomatis*. *New England Journal of Medicine*, 296, 306-310.

Frommell, G., Bruhn, F. W., and Schwartzman, J. D. (1977). Isolation of *Chlamydia trachomatis* from infant lung tissue. *New England Journal of Medicine*, 296, 1150-1152.

Experimental infections of the chimpanzee urethra and pharynx with *Chlamydia trachomatis*

N. F. JACOBS, E. S. ARUM, AND S. J. KRAUS (1978). *Sexually Transmitted Diseases* 5, 132-136

An isolate of *Chlamydia trachomatis* obtained from a man with nongonococcal urethritis was used to produce experimental urethral and pharyngeal infections in chimpanzees. After urethral inoculation of only 8×10^1 inclusion-forming units (IFU), infections were established in three of three animals; urethral discharges developed in two. The infections persisted for five to nine weeks. Larger inocula (7×10^2 and 1×10^5 IFU) produced pharyngeal infections in two animals. The third animal's pharynx was not infected by 1×10^5 IFU. Chlamydial complement-fixing antibodies increased significantly in sera of two of three animals. This study provides an animal model for study of mucosal infection by *C. trachomatis*. This relative resistance of the chimpanzee pharynx to infection parallels clinical observations in man.

Authors' summary

Serological response of patients with nongonococcal urethritis to causative organism of contagious equine metritis 1977

C. E. D. TAYLOR, R. O. ROSENTHAL, AND D. TAYLOR-ROBINSON (1979). *Lancet*, 1, 700-701

The presence of agglutinins to the causative organism of contagious equine metritis (CEM) in human serum has been confirmed. Agglutinins were found in the serum of 84 (37.6%) of 223 patients with nongonococcal urethritis (NGU), and in 12.5% of these patients there was a four-fold or greater rise in titre during the course of their illness. There was no evidence that

these agglutinins were the result of infection by chlamydiae or ureaplasmas. Certain patients with these agglutinins seemed to respond better to therapy with antibiotics to which the CEM bacterium is susceptible *in vitro* than did patients in whom these agglutinins were not found. The findings suggest that the CEM bacterium or a micro-organism related to it may be aetiologically involved in a proportion of patients with NGU. A search for such an organism in these patients is in progress.

Authors' summary

Clinical and microbiological investigation of men with urethritis

Y.-H. LEE, B. ROSNER, S. ALPERT, N. J. FIUMARA, AND W. M. MCCORMACK (1978). *Journal of Infectious Diseases*, 138, 798-803

Chlamydia trachomatis urethritis in men attending a venereal disease clinic: a culture and therapeutic study

K. T. RIPA, P.-A. MÅRDH, AND I. THELIN (1978). *Acta Dermatovenereologica*, 58, 175-179

Chlamydiae, cervicitis, and abnormal Papanicolaou smears

M. C. CARR, L. HANNA, AND E. JAWETZ (1979). *Obstetrics and Gynecology*, 53, 27-30

Chlamydial pneumonia and human sexuality (leading article)

R. B. GOLDBLOOM (1978). *Canadian Medical Association Journal*, 119, 1153-1154

Experimental acute salpingitis in grivet monkeys provoked by *Chlamydia trachomatis*

K. T. RIPA, B. R. MELLER, P.-A. MÅRDH, E. A. FREUNDT, AND F. MELSEN (1979). *Acta Pathologica et Microbiologica Scandinavica*, 87, 65-70

Cultivation of *Ureaplasma urealyticum* in continuous culture

G. MASOVER, R. PEREZ, AND A. MATIN (1979). *Infection and Immunity*, 23, 175

Trichomoniasis

Strain of *Trichomonas vaginalis* resistant to metronidazole and other 5-nitroimidazoles

J. G. MEINGASSNER AND J. THURNER (1979). *Antimicrobial Agents and Chemotherapy*, 15, 254-255

Candidosis

Vulvovaginal candidiasis in pregnancy treated with clotrimazole

K. HARAM AND A. DIGRANES (1978). *Acta Obstetrica et Gynecologica Scandinavica*, 57, 453-456

Genital herpes

Clinical course and diagnosis of genital herpes simplex virus infection and evaluation of topical surfactant therapy

L. A. VONTVER, W. C. REEVES, M. RATTRAY, L. COREY, M. A. REMINGTON, E. TOLENTINO, A. SCHWEID, AND K. K. HOLMES (1979). *American Journal of Obstetrics and Gynecology*, 133, 548-554

A prospective study of 37 men and 32 women with culture-proved genital herpes simplex virus (HSV) infection was undertaken to characterise the clinical manifestations of the disease and effects of topical therapy with Nonoxynol 9, a non-ionic surfactant active *in vitro* against HSV. The duration of pain, lesions, and viral shedding was significantly longer in initial than in recurrent genital HSV infection. Cervical HSV shedding occurred in 11 of 13 initial infections versus two of 19 recurrences ($P < 0.001$). Type 1 HSV caused seven of 29 initial and none of 40 recurrent infections ($P < 0.01$). Clinical recurrences within six weeks were detected more often in men than in women and were unrelated to the menstrual cycles. The influence of coitus on recurrences is unclear. Papanicolaou smears were transiently abnormal (Class II) in four (12.5%) of 32 female patients. Evidence of herpesvirus was detected in cytological smears from only 28 (41%) of 69 culture-proved external lesions and three (23%) of 13 culture-

positive cervixes. Serology was also of limited diagnostic value except in testing paired sera from initial infections. The topical therapy had no beneficial effect.

Authors' summary

Treatment of genital herpes simplex virus infection with photodynamic inactivation

R. H. KAUFMAN, E. ADAM, R. R. MIRKOVIC, J. MELNICK, AND R. L. YOUNG (1978). *American Journal of Obstetrics and Gynecology*, 132, 861-869

Herpes virus fingerprinting (leading article) *Lancet*, 1979, 1, 251-252

Other sexually transmitted diseases

Epidemic of chancroid in Greenland 1977-1978

T. LYKKE-OLESEN, T. G. PEDERSEN, L. LARSEN, AND K. GAARSLEV (1979). *Lancet*, 1, 654-655

In Greenland there has been a rapid increase in the incidence of gonorrhoea and syphilis during past decades. In 1977 there was an epidemic of chancroid along the west coast of Greenland, with 975 cases reported from some 32 500 adults. The number of reported cases increased until October, 1977, and subsequently decreased. A total of 186 patients was studied. Many of these had previously had gonorrhoea and syphilis. Male patients were both Eskimoan and Danish but female patients were solely Eskimoan. The sex ratio (M/F) was 1.6:1. The incubation period was four days in men and 13 days in women. Symptom-free female carriers did not seem to be an important reservoir of infection. Fifteen per cent of the patients were admitted to hospital with buboes or extensive lesions. The clinical course was uncomplicated in most cases. Ulcers healed within a week of treatment with sulphonamide. Chancroid can be expected to disappear in Greenland within a short time.

Authors' summary

Human wart virus antibodies in patients with genital and skin warts

S. PYRHÖNEN (1978). *Acta Dermatovenereologica (Stockholm)*, 58, 427-432

A comparative follow-up study of the

antibody response against human wart-virus was performed using the immunodiffusion and complement fixation methods on patients with condylomas and skin warts. By the immunodiffusion method, 13% of the patients with skin warts and 3% of the patients with condylomas showed >fourfold increase of antibody titre during the follow-up of 2-35 months. The findings show some typical features of the weak antibody response of a chronic viral infection and suggest a serological overlapping between condyloma viruses and certain group of skin wart-viruses. The antibody prevalence in age-matched controls is shown to be significantly higher than that in the wart or condyloma patients' initial serum samples. This is indicative of the protective function of antibodies against warts and condylomas. Also the analysis of the history of warts in patients with condylomas suggests that protection against condylomas can be acquired from previous warts, evidently by immunological mechanisms. In a control group of medical students, human wart-virus antibodies were frequently (52%) found in subjects without any history of warts. This finding supports the view that human wart-virus can frequently induce latent or subclinical infections in human beings.

Author's summary

The beneficial effects of 1% 5-fluorouracil in 70% ethanol on therapeutically refractory condylomas in the preputial cavity

G. VON KROGH (1978). *Sexually Transmitted Diseases*, 5, 137-140

The effect of two daily applications of 1% 5-fluorouracil (5-FU) in 70% ethanol on penile condylomas was investigated in 65 men. Complete cures were obtained in 37% and marked regression in 42% of the patients. The most pronounced effect was seen on warts in the preputial cavity and on the adjacent foreskin, where the efficacy of 1% 5-FU in ethanol was comparable to that of 5% 5-FU cream. However, the pronounced side effects observed with use of the 5-FU cream were reduced to negligibility by use of an alcoholic vehicle for the drug. Patients who have therapeutically refractory condylomas in or near the preputial cavity should receive one or more courses of treatment with 1% 5-FU in ethanol. With regard to meatal warts, the effect of two daily applications of 5% 5-FU cream for two weeks was found to be

therapeutically inferior compared with results of previous studies in which the same treatment was extended for three to four weeks.

Author's summary

Liver disease among homosexual males

W. R. ELLIS, I. M. MURRAY-LYON, J. C. COLEMAN, B. A. EVANS, J. L. FLUKER, J. BULL, P. W. N. KEELING, P. D. SIMMONS, J. E. BANATVALA, J. R. WILLCOX, AND R. P. H. THOMPSON (1979). *Lancet*, 1, 903-905

A survey of 2612 homosexual men for the presence of hepatitis B surface antigen (HBsAg) yielded positive results in 129 (4.94%) of the men. This incidence is 50 times greater than that found in blood donors in the United Kingdom. Sixty-five of the HBsAg-positive patients were tested for abnormal liver function tests (LFTs), of which 43 proved to have impairment. Twenty-five of the abnormal LFT group had a liver biopsy, of which 14 showed chronic active hepatitis and the remaining 11 minor inactive liver disease. Of 118 HBsAg-positive males examined for e antigen (HBeAg), 38% had positive results. There was no correlation between HBeAg-positive patients and chronic active hepatitis, over half the patients with inactive liver disease having positive results for this antigen. This result contrasts with a report by others of 13% of HBsAg-positive heterosexual men being also HBeAg-positive.

The important recommendation of this paper is that HBsAg-positive patients with abnormal liver function tests should be subjected to liver biopsy. By implication, it would appear that all admitted homosexual men should be tested for HBsAg on attendance.

G. L. Ridgway

Antibodies against chlamydiae of lymphogranuloma-venereum type in Crohn's disease

J. L. SCHULLER, J. PIKET-VAN ULSEN, I. V. D. VEEKEN, M. F. MICHEL, AND E. STOLZ (1979). *Lancet*, 1, 19-20

Antibodies against *Chlamydia* of lymphogranuloma venereum type were detected by the microimmunofluorescence technique in 38 (69%) of 55 patients with Crohn's disease. Weak positive reactions were seen in only two of 21 patients with other gastrointestinal disorders and in one of 50 healthy blood donors.

Authors' summary

Low frequency of chlamydial antibodies in patients with Crohn's disease and ulcerative colitis

D. TAYLOR-ROBINSON, C. S. O'MORAIN, B. J. THOMAS, AND A. J. LEVI (1979). *Lancet*, **1**, 1162-1163

Serum samples from 55 patients with Crohn's disease and from 23 patients with ulcerative colitis were tested for antibodies to *Chlamydia trachomatis* immunotypes by micro-immunofluorescence technique. Antibody titres of 1/8 or greater against several immunotypes were detected in 14.5% of patients with Crohn's disease and in 21.7% of those with ulcerative colitis. These figures resemble the incidence in a healthy, non-venereal disease population. Furthermore, there was no correlation between the presence of antibody and such factors as duration of symptoms, localisation of disease, or disease activity. These findings indicate that there is no reason to believe that Crohn's disease involves chlamydiae or that examination for chlamydial antibody is helpful in diagnosis.

Authors' summary

Salient features of *Haemophilus vaginalis*

J. R. GREENWOOD AND M. J. PICKETT (1977). *Journal of Clinical Microbiology*, **9**, 200-204

Factors affecting isolation and identification of *Haemophilus vaginalis* (*Corynebacterium vaginale*)

R. K. BAILEY, J. L. VOSS, AND R. F. SMITH (1979). *Journal of Clinical Microbiology*, **9**, 65-71

These two papers deal with the laboratory aspects of isolation and identification of *Haemophilus vaginalis*.

Greenwood and Pickett tested 78 strains and give recommendations for laboratory identification. Bailey *et al.* report on isolation procedures, finding that anaerobiosis is not required for primary isolation and that a six-hour delay before plating does not reduce the isolation rate.

This paper also assesses the behaviour of this organism in some of the commercially available identification systems.

These papers are of interest to those laboratory workers who wish to differentiate *H. vaginalis* from other Gram-negative vaginal flora.

M. C. Kelsey

Bacteriology of acute pelvic inflammatory disease

A. W. CHOW, V. PATTEN, AND J. R. MARSHALL (1979). *American Journal of Obstetrics and Gynecology*, **133**, 362-365

The bacteriology of acute pelvic inflammatory disease (PID) was studied in 20 patients by simultaneous cul-de-sac, cervical, and blood cultures with aerobic and fastidious anaerobic techniques. Cul-de-sac cultures gave positive results in 18 of 20 patients while all eight normal control subjects yielded negative results ($P < 0.0005$, χ^2 analysis.). Anaerobic bacteria (predominantly *Peptococcus* and *Peptostreptococcus*) were present in 10 (59%) patients, and they were the exclusive isolates in three patients. *Neisseria gonorrhoeae* was isolated only once from the cul-de-sac despite its presence in cervical specimens. There was poor correlation between cul-de-sac and cervical cultures. Cervical cultures yielded a variety of aerobic and anaerobic bacteria with an average of 3.8 organisms per specimen. *N. gonorrhoeae* was isolated in 13 (65%) patients. Only 19% of cervical isolates were recovered by culdocentesis; similarly, only 31% of cul-de-sac isolates were simultaneously isolated from the cervix. Blood cultures were uniformly negative. These data suggest that: (1) culdocentesis is a reliable technique for the bacteriological diagnosis of acute PID, and (2) whereas the gonococcus may be important in initiating acute PID its primary role appears to be paving the way for secondary invaders from the normal vaginal flora to gain access to the upper genital tract.

Authors' summary

Genital mycoplasmas and birth weight in offspring of primigravid women

R. F. HARRISON, R. HURLEY, AND J. DELOUVOIS (1979). *American Journal of Obstetrics and Gynecology*, **133**, 201-203

The relationship between colonisation of pregnant women by *Ureaplasma* and *Mycoplasma* and the outcome of pregnancy in a study of 104 women is reported. There were eight abortions but no multiple births or stillbirths. Excluding abortions, the mean gestational length was 39.6 weeks and the corrected mean birth weight was 3.28 kg. There was no relationship between maternal colonisation by genital mycoplasmas and reduction in birth weight of the offspring, but five of the eight women who aborted harboured *Ureaplasma*, suggesting an increased trend toward abortion in women harbouring *Ureaplasma*.

Authors' summary

Sexually transmitted infections in homosexual men

R. R. BABB (1979). *Postgraduate Medicine*, **65**, 215-222

Natural history and prevention of hepatitis B re-examined

S. KRUGMAN, L. R. OVERBY, I. K. MUSHAHWAR, C.-M. LING, G. G. FROSNER, AND F. DEINHARDT (1979). *New England Journal of Medicine*, **300**, 101-105

Behcet's syndrome associated with pregnancy

W. G. HURT, C. L. COOKE, W. P. JORDAN, J. P. BULLOCK JUN., AND G. E. RODRIGUEZ (1979). *Obstetrics and Gynecology*, **55** (Supplement) 31S-33S

Penile venereal edema

R. A. WRIGHT AND F. N. JUDSON (1979). *Journal of the American Medical Association*, **241**, 157

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 - 2 Brit. J. Vener. Dis. (1975), 51, 206-209
 - 3 Brit. J. Vener. Dis. (1976), 52, 43-45
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Contents

Evaluation of microhaemagglutination assay to determine treponemal antibodies in CSF

A. BIRRY AND S. KASATTYA (Canada) *page 239*

Attachment of *Neisseria gonorrhoeae* to human sperm: Microscopical study of effect of trypsin and iron

C. I. GOMEZ, W. A. STENBACK, A. N. JAMES, B. S. CRISWELL, AND R. P. WILLIAMS (USA) *page 245*

Studies of ciliated epithelia of the human genital tract

P.-A. MÅRDH, B. BALDETORP, C. H. HÅKANSSON, H. FRITZ, AND L. WESTRÖM (Sweden) *page 256*

Secretory antibody response of the cervix to infection with *Neisseria gonorrhoeae*

A. McMILLAN, G. McNEILLAGE, H. YOUNG, AND S. S. R. BAIN (Gt Britain) *page 265*

Evaluation of urethral culture for *Neisseria gonorrhoeae* in the routine investigation of men attending a STD clinic

A. McMILLAN AND R. S. PATTMAN (Gt Britain) *page 271*

Gonorrhoea in women in Scotland

C. B. S. SCHOFIELD (Gt Britain) *page 274*

How often are gonorrhoea and genital yeast infection sexually transmitted?

R. N. THIN, P. RENDELL, AND J. WADSWORTH (Gt Britain) *page 278*

The need for a chlamydial culture service

J. R. WILLCOX, P. G. FISK, J. BARROW, AND D. BARLOW (Gt Britain) *page 281*

Double-blind comparison of two regimens in the treatment of nongonococcal urethritis: seven-day vs 21-day courses of triple tetracycline (Deteclo)

I. V. THAMBAR, P. D. SIMMONS, R. N. THIN, S. DAROUGAR, AND P. YEARSLEY (Gt Britain) *page 284*

Rapid staining technique for *Trichomonas vaginalis*

M. J. BALSDON, N. GREEN, C. W. ANDREW, AND D. H. JACKSON (Gt Britain) *page 289*

False-positive test results for syphilis in relatives of a patient with systemic lupus erythematosus

S. JESSOP AND P. BOTHA (South Africa) *page 292*

Social distribution of sexually transmitted diseases

P. M. BACON (Gt Britain) *page 295*

Sexually transmitted diseases in Ontario

R. D. CATTERALL (Gt Britain) *page 300*

Australian conference on STD in Perth, Western Australia, August 1978

R. S. MORTON *page 304*

Correspondence *page 305*

Book reviews *page 306*

Notice *page 306*

Abstracts *page 307*

44.
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October 1979 Vol. 55 No. 5

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¹Thin RN, Shaw EJ. Diagnosis of gonorrhoea in women. *Br J Vener Dis* 1979; 55: 224-225.

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²King A, Nicol CS. *Venereal Diseases*. 3rd ed. London: Baillière and Tindall, 1975.

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³Wentworth BB. Sensitivity of cell culture for isolation of *Chlamydia trachomatis* from genital sources. In: Hobson D, Holmes KK, eds. *Non-gonococcal Urethritis and Related Infections*. Washington DC: American Society for Microbiology, 1977: 214-222.

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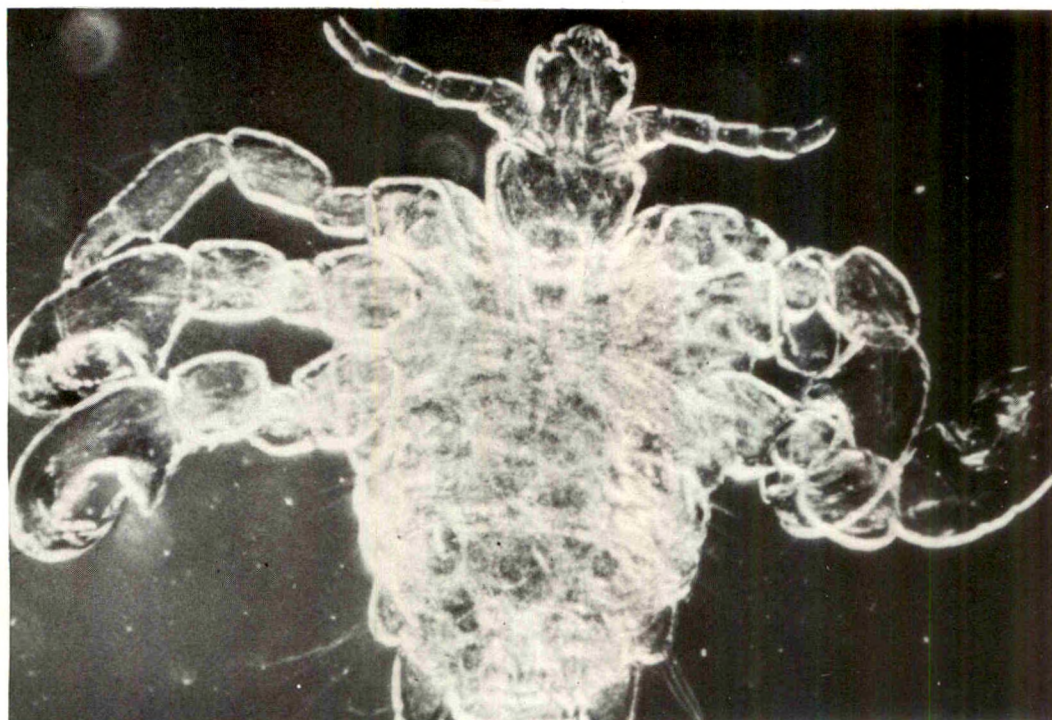
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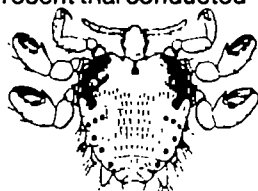
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1 Brit. J. Vener. Dis. (1975), 51, 97-98

2 Brit. J. Vener. Dis. (1975), 51, 206-209

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Study of STDs in patients attending venereal disease clinics in Khartoum, Sudan

O. M. A. TAHA, M. HAG ALI, E. E. OMER, M. A. AHMED, AND S. A. ABBARO

From the Department of Dermatology and Venereology, Omdurman and Soba University Hospital, Sudan

SUMMARY During the period October 1976 to January 1978, 290 patients were examined for sexually transmitted diseases in three venereal disease clinics in Khartoum Province. Clinical and laboratory findings showed that nongonococcal urethritis was the commonest STD in men (35.1%), with gonorrhoea the second commonest (25.9%). Most of the patients with STDs were aged between 20 and 39 years. Of the infected men, 49.3% had acquired their infections from prostitutes.

Introduction

Sexually transmitted diseases (STDs) are currently the commonest contagious diseases in the world; the World Health Organisation's statistics recorded 200 million cases of gonorrhoea and 50 million cases of syphilis for the year 1972 (WHO, 1972).

Although these diseases cause much morbidity, mental ill-health, and social problems, at present they are being neglected in developing countries. There are no facilities for correct diagnosis and treatment. No adequate statistics have been collected and no previous studies carried out to determine the size of the problem (Sogbetun *et al.*, 1977). This prompted us to report our experience of the pattern of these diseases as seen at three venereal disease clinics in Khartoum over a period of 15 months.

Patients and methods

Two hundred and ninety patients referred with a diagnosis of STD to the venereal disease clinics at Soba and Omdurman Hospitals and at the Saggana Health Centre by medical practitioners in different centres in Khartoum Province were studied over the period October 1976 to January 1978. Each clinic was held once a week.

All patients had samples of blood taken for the Venereal Disease Research Laboratory (VDRL) and fluorescent treponemal antibody-200 (FTA-200) tests. In patients with genital ulcers darkground examination of the exudate was performed. Urethral

specimens were collected, and the smears were stained by Gram's method and examined microscopically for leucocytes and intracellular Gram-negative diplococci. Specimens were also plated in the clinic on chocolate agar medium and incubated immediately at 37°C in a candle-extinction tin. Gonococci were identified by characteristic Gram-stain morphology, oxidase test, and sugar fermentation reactions.

Sabouraud's medium was inoculated for isolation of *Candida albicans*. Germ-tube and sugar fermentation tests were carried out for full identification of the organism.

A two-glass urine test was performed in male patients who presented with urethritis; if threads were noted a centrifuged deposit of urine was examined microscopically.

The Frei's test, lymphogranuloma venereum complement fixation test (LGVCFT), and examination of smears of ulcers for *Haemophilus ducreyii* were not performed for lack of facilities. The diagnosis of lymphogranuloma venereum (LGV), genital warts, molluscum contagiosum, and pediculosis pubis were made on their characteristic clinical features. Pediculosis pubis was diagnosed from the clinical features and after the louse of the *Pediculus pubis* had been identified under the microscope.

Results

Of the total of 290 patients referred with a diagnosis of STD to the three venereal disease clinics, 148 (51%) did not have an STD.

Only one case of primary syphilis was detected by darkground microscopy, and this was confirmed by

Address for reprints: Dr M. Hag Ali, Medical Research Council, PO Box 1304, Khartoum, Sudan

Received for publication 5 December 1978

the results of the VDRL and FTA-200 tests (Table 1). Other STDs included candidosis, trichomoniasis, gonorrhoea, nongonococcal urethritis (NGU), genital warts, LGV, molluscum contagiosum, pediculosis pubis, and non-syphilitic penile ulcers (Table 1). The cases of NGU included two patients who were found to have genital candidosis.

Table 1 *Analysis of the diagnoses*

Diagnosis	Men		Women		Total	
	No.	%	No.	%	No.	%
Trichomoniasis	0	0	23	32.4	23	15.5
Candidosis	0	0	43	60.6	43	29.1
Gonorrhoea	20	25.9	5	7.0	25	16.9
Nongonococcal urethritis	27	35.1	0	0	27	18.1
Syphilitic penile ulcers	1	1.3	0	0	1	0.7
Non-syphilitic penile ulcers	18	23.4	0	0	18	12.2
Warts	4	5.2	0	0	4	2.7
Lymphogranuloma venereum	4	5.2	0	0	4	2.7
Molluscum contagiosum	2	2.6	0	0	2	1.3
Pediculosis pubis	1	1.3	0	0	1	0.7
Total	77	100	71	100	148	100

The age range of all the patients according to diagnosis is shown in Table 2. Most of the male patients had sexual contact with prostitutes and girlfriends (Table 3).

Discussion

As in other parts of the world (Willcox, 1975; Sogbetun, 1977), we found that NGU was the most common STD among male patients (35.1%) followed by gonococcal urethritis (25.9%). Syphilis accounted for only 1.3% of patients. This low incidence of syphilis may be because patients with genital ulcers fear syphilis and seek antisyphilitic treatment at a very early stage at the nearest pharmacy, or from

Table 3 *Sexual contacts of male patients*

Contact	No.	%
Prostitute	38	49.3
Girlfriend*	5	6.6
Wife	0	0
Not admitted	34	44.1
Total	77	100

*Regular sexual relationship for at least three months but without legal marriage or dependent on financial payments or other gifts.

hospital orderlies, or from the doctor before reporting to the venereal disease clinic.

Candidosis was the commonest STD among female patients (60.6%), with most cases in the age group 20-29 years. Trichomoniasis was the second commonest condition, and again most cases were in the age group 20-29 years.

Most of our patients (86.5%) were aged between 20 and 39 years, which coincides with the period of greatest sexual activity and follows the same worldwide pattern observed by Idsoe *et al.* (1973).

Although prostitution is prohibited in Khartoum, 49.3% of the male patients were infected by prostitutes. This figure reflects the degree of inefficiency in enforcing this legislation. On the other hand, 44.1% of the male patients did not admit the source of their infection; they denied having any sexual intercourse and stated that they developed the STD after a nightmare. This illustrates the patients' ignorance concerning the transmission of these diseases, their lack of health education, and the difficulty to be expected when planning contact-tracing in the future. Contact-tracing is not carried out at present because of lack of facilities, lack of personnel, and other social factors.

We acknowledge the co-operation and technical assistance of Mr A. Darrag and other workers in the Department of Medical Microbiology, Faculty of Medicine, Khartoum.

Table 2 *Relationship of age to incidence of STDs*

Diagnosis	Age group (years)						Total
	15-19	20-29	30-39	40-49	50 and over	Not certain	
Trichomoniasis	1	10	8	2	1	1	23
Candidosis	1	20	14	6	0	2	43
Gonorrhoea	2	17	6	0	0	0	25
Nongonococcal urethritis	0	21	6	0	0	0	27
Penile ulcers	1	11	6	1	0	0	19
Genital warts	0	4	0	0	0	0	4
Molluscum contagiosum	0	1	1	0	0	0	2
Pediculosis pubis	1	0	0	0	0	0	1
Lymphogranuloma venereum	1	2	1	0	0	0	4
Total (%)	7 (4.7)	86 (58.1)	42 (28.4)	9 (6.1)	1 (0.7)	3 (2.0)	148 (100)

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Use of CB hamsters in the study of *Treponema pertenue*

RONALD F. SCHELL, JACK L. LE FROCK, JEGDISH P. BABU, AND JOHN K. CHAN

From the Department of Microbiology and Immunology and the Department of Medicine (Division of Infectious Diseases), Albany Medical College, and the Clinical Microbiology Laboratory, Albany Medical Center Hospital, Albany, USA

SUMMARY The CB/Ss LAK strain of inbred hamster was used as a model for studies of infection with *Treponema pertenue* and of acquired resistance to it. When infected, this strain developed cutaneous lesions which lasted for six to seven months, even in the presence of peak titres of antitreponemal antibody. The rate of appearance and resolution of these lesions varied with the size of the inoculum. The infected hamsters' inguinal lymph nodes increased significantly in weight and teemed with treponemes for several weeks. Animals infected for eight or 10 weeks obtained quick resolution of their lesions by treatment with penicillin and were thereafter resistant to reinfection.

Introduction

For investigations of the immune mechanism of frambesia (yaws), inbred strains of hamsters are becoming recognised as the animal model of choice. Two such studies with inbred golden hamsters have been reported (Guerraz *et al.*, 1977; Schell *et al.*, 1978).

We have found that CB/Ss LAK (CB) strain of hamster responds especially rapidly to infection with *Treponema pertenue*, the causative agent of frambesia, and that pathological changes induced in this strain can be readily quantitated. For these reasons we are now using the CB strain in experimental studies of passive transfer of resistance to *T. pertenue*.

The advantages of the hamster model—and of the CB strain in particular—are presented in this report.

Materials and methods

ANIMALS

Five inbred hamster strains were studied initially: CB, MHA/Ss LAK, LSH/Ss LAK, PD4/LAK, and LHC/LAK. These were obtained from Charles River Breeding Laboratories Inc., Wilmington,

Massachusetts. Hamsters weighing 80-100 g were housed five or six per cage at an ambient temperature of 18°C, a condition which facilitates the development of cutaneous lesions (Hollander and Turner, 1954).

ORGANISM

T. pertenue strain Haiti B (obtained from Paul H. Hardy, Jr., Johns Hopkins University) was maintained by passage in hamsters. The inguinal lymph nodes were removed aseptically three to four weeks after intradermal infection, teased apart in sterile saline, and filtered through 60-mesh steel wire. After centrifugation at 270 × g for three minutes to remove cellular debris, the number of treponemes in the supernatant was determined by darkfield microscopy.

COUNTING OF TREPONEMES

The approximate number of treponemes per lymph node was determined according to the procedure described by Miller (1971). Briefly, duplicate slides of each homogenised lymph node were prepared and 120 fields per slide were examined for treponemes by darkfield microscopy.

SEROLOGICAL TEST

The Sera-Tek treponemal antibody test (manufactured by Fujizoki Pharmaceutical Co. Ltd., Shinjuku-ku, Tokyo, Japan) was used. The test was

Address for reprints: Dr Ronald F. Schell, Clinical Microbiology Laboratory, Albany Medical Center Hospital, Albany, NY 12208, USA

Received for publication 28 February 1979

performed as specified by the manufacturer, except that sera obtained from frambesial hamsters were serially diluted with absorbing diluent to yield quantitative titres.

STATISTICAL ANALYSIS

Fischer's least significant difference test (Steel and Torrie, 1960) was used to examine pairs of means when a significant F ratio indicated reliable mean differences. The alpha level was set at 0.05 before the experiments started.

Results

APPEARANCE OF CUTANEOUS LESIONS

Ten hamsters from each of the five strains were infected intradermally in the inguinal region with 1×10^6 *T. pertenu* and examined weekly for development of cutaneous lesions.

Three weeks after infection lesions were found in all CB hamsters, in one MHA/Ss LAK hamster, and in none of the remaining strains (Table 1). Although more lesions developed in each of the other strains up to 12 weeks after infection, in only the CB strains were all test animals affected. Similar results were obtained when this study was repeated, and no new lesions occurred in any strain after 12 weeks. All further experiments were conducted with the CB strain.

Table 1 Cumulative number of hamsters from five inbred strains developing cutaneous lesions

Strain (n = 10)	Weeks after infection			
	3	5	8	12
CB/Ss LAK	10	10	10	10
MHA/Ss LAK	1	6	8	8
LSH/Ss LAK	0	3	3	5
PD4/LAK	0	2	3	7
LHC/LAK	0	5	7	7

EFFECT OF INOCULUM SIZE

Groups of six CB hamsters were inoculated intradermally with 1×10^2 , 1×10^3 , 1×10^4 , 1×10^5 , or 1×10^6 treponemes. The rate of development of cutaneous lesions was directly dependent on the number of treponemes injected (Table 2). All hamsters given $\geq 1 \times 10^4$ treponemes, however, formed lesions within six weeks. In groups given $\leq 1 \times 10^3$ treponemes, few or no animals showed lesions even after observation for 12 weeks.

Hamsters inoculated with 1×10^5 or 1×10^6 treponemes developed extensive, chronic, skin lesions, which usually resolved six to seven months after infection. In a few (10%) hamsters the lesions remained nine months after infection. In contrast,

Table 2 Cumulative number of CB hamsters developing cutaneous lesions after varying inocula of *T. pertenu*

Weeks after infection	Inoculum (six animals/group)				
	10^6	10^5	10^4	10^3	10^2
1					
2	1				
3	6				
4	6	4	1		
5	6	6	2		
6	6	6	6	2	

lesions regressed rapidly in hamsters inoculated with 1×10^3 or 1×10^4 treponemes. Thus inoculum size influenced not only the development but also the resolution of cutaneous lesions.

LYMPH NODE WEIGHTS AND TREPONEMAL COUNTS

Thirty-six hamsters were infected in the inguinal region with 1×10^6 *T. pertenu*. At weekly intervals for 12 weeks three frambesial hamsters were bled by cardiac puncture and killed. The inguinal nodes were removed aseptically, and the approximate number of treponemes per node was determined by darkfield microscopy. Three age-matched, non-infected hamsters were killed each week to determine the weights of normal inguinal lymph nodes.

The inguinal nodes from frambesial hamsters rapidly increased in weight, reaching a peak five weeks after infection (Figure). The number of treponemes detected in the lymph nodes increased concomitantly (Table 3). Six weeks after infection the

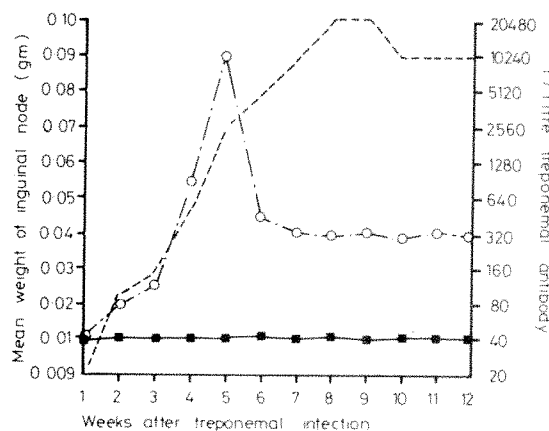


Figure Mean weights of inguinal lymph nodes in normal CB hamsters (■) and in hamsters infected with 1×10^6 *T. pertenu* (○). (Standard error for each mean = 0.009). Broken line shows antitreponemal antibody response in infected animals.

Table 3 *Approximate number of treponemes in inguinal lymph node after infection with T. pertenu*

Week after infection	Mean No. of treponemes $\times 10^{**}$
1	5.1
2	58.8
3	729.0
4	996.0
5	1610.0
6	633.0
7	209.0
8	131.0
9	128.0
10	111.0
11	98.0
12	120.0

*Three hamsters per group interval

weight decreased by 50% and the number of treponemes declined sharply.

ANTIBODY RESPONSE

Antitreponemal antibody titre increased slowly during the first three weeks after infection (Figure). Thereafter the titre increased rapidly, reaching a peak eight to nine weeks after infection. Infected hamsters retained this peak titre nine months after infection. When the experiment was repeated, similar results were obtained.

RESISTANCE TO REINFECTION

At two-week intervals three groups of six hamsters each were infected intradermally with 1×10^6 *T. pertenu*. At week 10 (that is, after six, eight, or 10 weeks of infection) 3000 units of penicillin were administered to all three groups plus a fourth group of six non-infected hamsters. Ten days later all four groups were challenged intradermally with 1×10^6 *T. pertenu*.

Lesions developed after 17 ± 2 days in the controls and after 28 ± 3 days in animals which had previously been infected for six weeks. In contrast, no cutaneous lesions were detected even after prolonged observation (total seven weeks) in hamsters that had been infected previously for eight or 10 weeks.

Twenty-eight days after reinfection three hamsters from each group were killed. No treponemes were detected in the lymph nodes of hamsters previously infected for eight or 10 weeks. A large number of treponemes was detected in the lymph nodes of controls (2.3×10^7) and of hamsters previously infected for six weeks (1.2×10^6). The weight of the lymph nodes averaged 0.06 g in controls, 0.04 g in animals previously infected for six weeks, and 0.01 g in animals previously infected for eight or 10 weeks (standard error for each mean = 0.009). The reduction in weight in the latter groups (eight or 10

weeks) compared with that in the controls was statistically significant ($P < 0.001$).

These results showed that after eight weeks of frambesial infection the hamsters had developed an effective immune response.

Discussion

For experimental studies of the immune mechanism of frambesial infection, hamsters have several distinct advantages:

- (1) Inbred CB hamsters are readily available and are required for immunological studies of the way resistance may be transferred to normal recipients with sera or cells from animals immune to frambesial infection.
- (2) Infection of inbred hamsters with *T. pertenu* consistently produces extensive, chronic, skin lesions that resemble human frambesial infection (Williams, 1935).
- (3) Frambesial lymph nodes can be easily detected in hamsters, and their increased weight is a useful measure of pathogenicity and infectivity.
- (4) Since the lymph nodes of frambesial hamsters teem with treponemes their numbers can be readily estimated by darkfield microscopy.
- (5) The hamster has no known treponemal disease that might influence an experimental infection (Willcox and Guthe, 1966).
- (6) The inbred hamster is relatively inexpensive and does not require elaborate animal facilities.

The ultimate goal of treponemal research is to develop an effective vaccine. For this purpose it is especially important that treponemal infections of vaccinated and non-vaccinated inbred hamsters can be compared in three parameters: (1) lymph-node weight, (2) number of treponemes per inguinal lymph node, and (3) development of cutaneous lesions. In the frambesial rabbit model only the cutaneous lesions (Eagle, 1948) can be readily measured.

Although Geiman and McKee (1950) and several other investigators (Guimaraes, 1953; Rosenau, 1953; Hill and Gordon, 1954; Turner and Hollander, 1957; Blom *et al.*, 1976; Hovind-Hougen *et al.*, 1976) have shown that outbred hamsters can be infected with *T. pertenu*, only one inbred strain capable of such infection has been reported. These are the golden hamsters used by Guerraz *et al.* (1977). Our results show that the CB hamster is also an excellent model for treponemal studies. When infected, it regularly developed cutaneous lesions, which normally persisted for six to seven months, even in the presence of peak anti-treponemal antibody titres. The rate of appearance of cutaneous lesions and of

their resolution varied measurably with the size of the inoculum. In addition, the infected CB hamster's inguinal lymph nodes increased significantly in weight and teemed with treponemes for several weeks. Similar results were reported for outbred strains by Geiman and McKee (1950), Turner and Hollander (1957), and Rosenau (1953).

The cutaneous lesions resolved rapidly after treatment with penicillin. When CB hamsters are so treated after eight or 10 weeks of infection and are subsequently reinfected with *T. pertenu*, no frambesial lesions develop. The ability to acquire resistance to reinfection makes this strain an appropriate one for studies of passive transfer of resistance, which are now under way in our laboratory (Schell *et al.*, 1978).

Our thanks to Bruce Calman and Barbara Wells for technical help and to Diane Sager for secretarial assistance. This investigation was supported by the World Health Organisation and United States Public Health Service Research Grant AL 13307. Dr John K. Chan is a fellow supported by the Venereal Diseases Research Fund of the American Social Health Association.

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Cultivation of *Treponema pallidum* in subcutaneous chambers implanted in golden hamsters

G. D. MORRISON,* S. I. EGGLESTONE,† AND J. L. NORTHWOOD‡

From the *Venereology Department and ‡Public Health and Microbiology Laboratory, Plymouth General Hospital; and †the School of Environmental Sciences, Plymouth Polytechnic, Plymouth

SUMMARY *Treponema pallidum* was successfully grown in subcutaneous chambers in golden hamsters. Abnormally long forms of *T. pallidum* were found in these chambers. The cause of this abnormality may possibly be due to their being coated with antibody which could inhibit cell division or render the organism avirulent or both. *T. pallidum* was successfully passaged between hamsters but with a possible diminution in virulence.

Introduction

The technique of growing *Treponema pallidum* in a chamber implanted in a host animal was described by Levaditi and McKintosh (1908). These authors used collodion sacs which were inserted into the peritoneal cavities of cynomolgous monkeys and rabbits. Secondary infection in the sacs rendered the technique unusable. The method was improved by Arko (1973), who used a plastic practice golf ball to form a subcutaneous chamber in the back of a rabbit. Tight and Perkins (1976) used this system to study the growth of *T. pallidum* in the rabbit. They also showed that the administration of corticosteroids increased the yield in the chamber but that the administration of oxisuran, which inhibits cell-mediated immunity, did not do so. A disadvantage of these chambers in the rabbit is the formation of chancres at the site of entry of needles used to sample the chamber contents, with the risk of the overlying skin breaking down. Rathlev (1973) used chambers made of small lengths of polythene tubing implanted under the skin of guinea pigs in which *T. pallidum* could be grown.

As we were interested in the development of a system which might isolate *T. pallidum* from body fluids, we originally tried this technique in albino rats but were unable to grow *T. pallidum* in them. Another drawback was the development of fibrosarcomas around the chambers, presumably due to carcinogens in the plastic. We then attempted the same technique in golden hamsters (*Mesocricetus*

auratus) and report here the successful growth in, and passage between, these chambers. The golden hamster was chosen because it is known not to have a naturally occurring treponemal infection and also because Turner and Hollander (1957) had shown that hamsters can carry *T. pallidum* over long periods.

Materials and methods

INSERTION OF CHAMBERS

Golden hamsters were anaesthetised with a mixture of halothane and air and placed prone on the operating table. The upper half of the back was clipped free of hair and the skin thoroughly disinfected. A midline incision, approximately 1-cm long, was made just posterior to the scapulae. Spencer Wells forceps were passed subcutaneously in a posterolateral direction to form a pocket in each flank and a sterile chamber inserted in each pocket using an aseptic technique. The skin incision was closed with Michel clips and the wound sprayed with a clear plastic dressing (Nobecutane, Astra Ltd.). The chambers were made of 2.5-cm lengths of Eschman polyvinyl chloride conductive tubing, internal bore 0.5 cm, and sterilised by autoclaving. The total volume of these chambers is 0.5 ml but the recoverable volume is 0.3 ml. After seven days the clips were removed under a light anaesthetic, and all subsequent procedures were performed on anaesthetised animals to prevent the operator being bitten. The hamsters were not used for at least 14 days, during which time the chambers filled with a clear fluid which on electrophoretic analysis showed only albumen and β -globulins to be present (Fig. 1). The animals were housed individually in cages at an environmental temperature of 60-70°F, fed on an

Address for reprints: Dr G. D. Morrison, Treatment Clinic, Plymouth General Hospital, Freedom Fields, Plymouth, Devon

Received for publication 19 March 1979

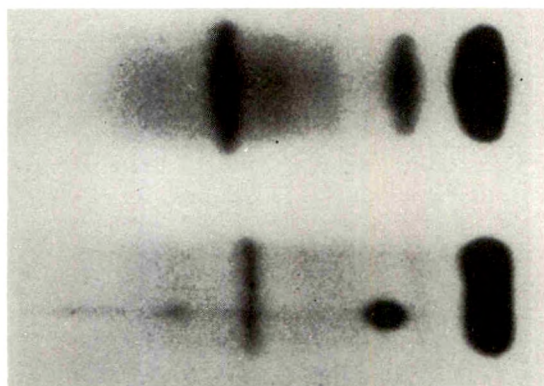


Fig. 1 Electrophoretic strips: upper strip = serum; lower strip = chamber fluid

antibiotic-free, commercially prepared, rodent food and given an unlimited amount of water.

SOURCE OF *T. PALLIDUM*

An initial intratesticular inoculation of *T. pallidum* (Nichols strain) in a rabbit produced the typical orchitis. The rabbit was killed and the testes removed. The *T. pallidum* was extracted from the testicular tissue as described by Wilkinson (1972). The treponemes were suspended in pH 7.4 phosphate-buffered saline and diluted to give a count of approximately 20 organisms per high power field using a $\times 100$ oil immersion objective and darkground illumination.

INOCULATION

Three experimental groups of hamsters (A, B, and C) were used and 0.02 ml of the treponeme suspension was injected into the chamber of each animal. The skin over one end was disinfected with 70% alcohol, and a fine needle (25 G) was inserted. Only very small quantities were withdrawn (enough to give one drop on a microscope slide) to avoid emptying the chamber. In addition to the treponeme suspension the hamsters in group B received an intramuscular injection of 0.02 ml methylprednisolone (Depo Medrone, Upjohn) in their thighs and group C an intramuscular injection of 0.02 ml of a long-acting triamcinolone (Kenalog, Squibb) to lengthen the duration of the immunosuppressive activity.

In a fourth group of hamsters, which were not inoculated with treponemes, chamber fluid was examined before and after treatment with long-acting triamcinolone to determine if naturally occurring treponeme-like forms occurred in the chambers.

MICROSCOPY AND SEROLOGY

Samples of fluid were withdrawn from the chambers at weekly intervals. One drop was placed on a slide

and 20 fields were counted using darkground illumination and a $\times 100$ objective. Passages between hamsters were performed by injecting 0.01 ml into the chamber of the recipient hamster.

Samples of chamber fluid were further examined by silver-staining, using Dunoyer's modification of Dieterle's method (Collart *et al.*, 1962) and by fluorescent antibody staining using an indirect method with known positive human serum (Venereal Disease Research (VDRL) test titre 1/32) from a patient with secondary syphilis and fluorescein conjugated anti-human globulin (Wellcome Laboratories Ltd).

Serological testing of chamber fluid and blood from the hamsters was performed when the animals were killed. We found that cardiac puncture caused too high a fatality rate to be performed more than once. The tests used were the *T. pallidum* haemagglutination assay (TPHA), the VDRL test, and the fluorescent treponemal antibody (FTA) test using anti-hamster globulin (Difco Laboratories) with Wellcome FTA antigen.

Results

The hamsters tolerated both the chambers and the repeated anaesthetics with no apparent ill effects. Post-mortem examination of hamsters whose chambers had been inoculated with *T. pallidum* did not show any lymphadenopathy, and histological examination of the skin overlying the end of the chamber showed no inflammatory response but only fibrous tissue with iron deposits, presumably from bleeding when the chamber was inserted.

The results of the attempts to grow *T. pallidum* in the chambers is shown in Table 1 and 2 and subsequent passage experiments in Table 3. Motile treponemes of normal length seen in the chamber fluid had the characteristic corkscrew morphology of *T. pallidum* (Fig. 2). Double-length organisms about to divide were commonly seen. In addition organisms between three and eight times their normal length were found. They showed diminished motility and less pronounced undulations (Fig. 3). These originally appeared eight weeks after the initial inoculation in hamsters treated with triamcinolone (group C) but not in those initially treated with methylprednisolone (group B) or untreated controls (group A). Subsequent passages from group C animals on all further occasions have shown both short and long forms in the chambers of the recipient hamsters. The ratio between these two forms was not constant, and there were times when the long forms disappeared and only normal-length forms were seen. Both normal and long forms showed positive fluorescent-antibody staining. Uninoculated

Table 1 Results of inoculation of hamster chambers

	Non-steroid treated							Steroid treated						
	Group A							Group B				Group C		
	1	2	3	4	5	6	7	1	2	3	4	1	2	3
Approximate latent period (days) before <i>T. pallidum</i> found in chamber														
1-7														
8-14				+				+	+					
15-21	+	+	+		+		+			+		+	+	+
22-28						+					+			
Length of time (days) <i>T. pallidum</i> present in chamber fluids														
1-7						+	+	+	*					
8-14		+	+	+	*								+	
15-21	+									+	*			
22-28														
29-35					+					+			+	+
>35										+				
Highest no. of <i>T. pallidum</i> in 20 fields at any time														
1-20		+				+	+	+	+	+	+			
21-40	+		+										+	
41-60				+	+									
>100													+	+

+ *T. pallidum* present

* Animal found dead or killed

Table 2 Results of serological tests

Site	Test*	Non-steroid treated							Steroid treated						
		Group A							Group B				Group C		
		1	2	3	4	5	6	7	1	2	3	4	1	2	3
Chamber fluid	TPHA	160	320	80	ND	—	—	—	ND	—	—	—	—	—	ND
	VDRL	1	4	2	ND	—	—	—	ND	—	—	—	—	—	ND
Serum	TPHA	320	640	—	ND	—	—	—	ND	—	—	—	—	—	ND
	VDRL	8	32	4	ND	—	—	—	ND	—	—	—	—	—	ND

— Negative

ND = not done

* Reciprocal titre

Table 3 Results of passage experiments

Hamster-to-hamster passage level	Donor-hamster no.	Days <i>T. pallidum</i> held in chambers before passage	Recipient-hamster no.	Latent period before <i>T. pallidum</i> found in chamber following passage	No. of days <i>T. pallidum</i> detected in recipient-hamster	Total time (days) <i>T. pallidum</i> held in hamster chambers
First	H1	13	H3	20	7	40
	H2	22	H4	14	22	58
	H2	22	H5	28	114	164
	H2	22	H6	28	165	215
Second	H5	77	H7	14	70	161
	H5	158	H8	7	28	193
	H6	77	H9	NF	0	77
	H6	188	H10	NF	0	188
Third	H7	98	H11	28	>197	>323
Fourth	H11	161	H12	NF	0	161
	H11	161	H13	21	28	210

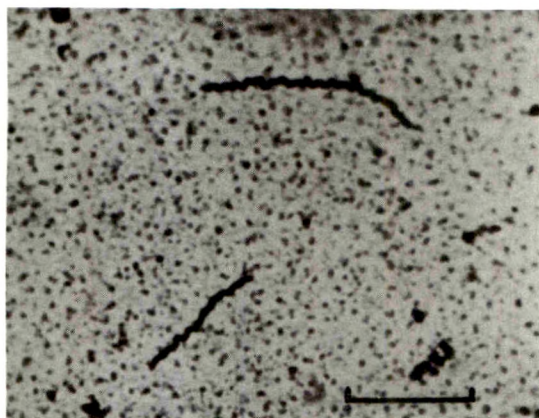


Fig. 2 Normal length treponemes as seen in chamber fluid. (Stained by Dunoyer's modification of Dieterle's method; internal scale marker = 10 μ)

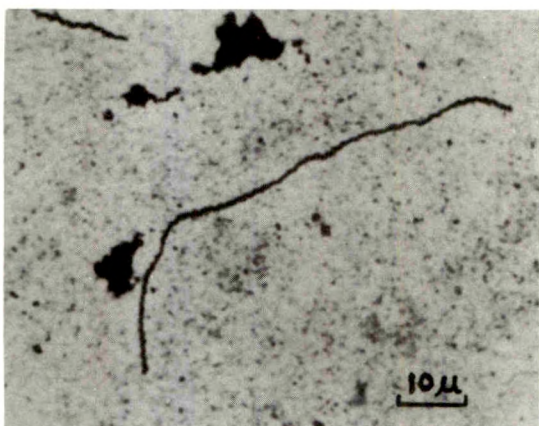


Fig. 3 Long form of treponemes as seen in chamber fluid. (Stained by Dunoyer's modification of Dieterle's method; internal scale marker = 10 μ)

hamsters, whether or not they had been treated with steroids, showed no organisms resembling treponemes in their chamber fluids.

Although the size of the drop of chamber fluid examined on different occasions varied slightly, the treponemes seemed to increase in numbers after a latent period during which no *T. pallidum* could be seen.

Chamber fluid containing long and short forms of *T. pallidum* was inoculated under the shaved neck of two rabbits with known negative serology; neither rabbit developed a chancre nor did it convert its serology during the subsequent three months.

Furthermore, direct-staining of the long forms using Difco anti-hamster fluorescein conjugate showed weak fluorescence.

Discussion

The hamster is known not to have a naturally occurring treponemal infection, and we were also unable to demonstrate treponeme-like forms in the chamber fluids of uninoculated controls with or without the administration of steroids. However, intradermal inoculation of hamsters with *T. pallidum* produces a generalised lymphadenitis with numerous treponemes (Turner and Hollander, 1957). As these changes were not seen in inoculated hamsters in the present study, it would suggest that encapsulation of the chamber takes place. The encapsulation could also explain, owing to lack of antigenic stimulus, the low systemic immune response observed.

The chamber system used was shown to support the growth of *T. pallidum* and it also showed that passage between hamster chambers could be achieved. However, many of the treponemes were clearly different in their morphology and virulence. Furthermore, the long forms showed some evidence of being coated with antibody, and it is possible that this antibody could inhibit cell division or render the organism avirulent or both. One cannot rule out the possibility that the organisms may have encountered deficiencies in essential metabolites while growing in the chambers, which may have affected their morphology and virulence.

We cannot explain at the present time the apparent inconsistencies of the immunological responses in the non-steroid treated group. Work is continuing in an attempt to elucidate these problems.

We wish to thank Dr A. E. Wilkinson and Mr G. Lavender of the Venereal Disease Reference Laboratory for supplying the *Treponema pallidum*, Dr P. J. L. Sequeira for helpful advice, Mrs M. Ashworth for attending to the welfare of the animals, Dr P. D. Meers and Dr G. Churcher for making available the facilities of the Plymouth Laboratory of the Public Health Laboratory Service, Dr J. Went for the electrophoretic studies, Dr A. C. Hunt for histological examinations, and Miss C. Ellis for typing the manuscript.

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Sensitivity of *Neisseria gonorrhoeae* to antibiotics in London (1976-78)

A. D. SETH, B. KOLATOR, AND A. E. WILKINSON

From the Venereal Diseases Reference Laboratory (PHLS), London Hospital Research Laboratories, London

SUMMARY Three surveys of the sensitivity of pretreatment isolates of gonococci to penicillin, ampicillin, tetracycline, spectinomycin, streptomycin, and co-trimoxazole were carried out during the period 1976-78. Compared with the results of previous surveys in the London area the decline in the proportion of strains of gonococci which are insensitive to penicillin appears to have halted. No changes were found in the levels of resistance to ampicillin, tetracycline, or spectinomycin during the period surveyed, although there was a slight increase in the proportion of strains requiring concentrations of 20 µg spectinomycin per ml for inhibition. Streptomycin resistance has decreased considerably and 91% of strains are now sensitive to 10 µg streptomycin per ml.

Introduction

The emergence of strains of *Neisseria gonorrhoeae* in the middle 1950s which were relatively insensitive to penicillin was rapidly followed by an increase in their prevalence; this trend was more marked in the United States than in the United Kingdom. Martin *et al.* (1970) found that only 0.6% of American strains isolated before 1955 had minimum inhibitory concentrations (MICs) greater than 0.03 µg/ml; in 1965 this figure had risen to 42% and between 1968 and 1969 to 65%. In a national survey carried out from 1972 to 1974 on 4405 isolates from 10 major American cities 70% of the strains had an MIC of ≥ 0.06 µg/ml, a figure almost identical with that of a previous survey carried out from 1970 to 1971 (Jaffe *et al.*, 1976). Marked differences were found in the proportion of the most resistant strains with an MIC of ≥ 0.5 µg/ml in different areas, from 7% in Des Moines to 26.6% in Denver; the overall figure for the whole survey was 17.4%.

More recently, penicillin resistance among gonococci appears to have shown a marked decline in the United States, from 53.2% of strains with an MIC of 0.25 µg/ml in 1972 to 29.2% in 1975 (Reynolds *et al.*, 1976). A similar rise and fall in the proportion of resistant strains has been found in Denmark but resistance is said to have increased during 1976 (World Health Organisation, 1978). The

appearance of β -lactamase-producing strains of gonococci early in 1976 (Ashford *et al.*, 1976) suggested that the favourable trend of decreasing resistance of gonococci to penicillin might be halted, but with the exception of the localised outbreak in Liverpool between 1976 and 1977, which was rapidly contained (Arya *et al.*, 1978), these strains do not appear to have been able to establish themselves in Europe or America.

Although sensitivity studies on nationally collected strains provide an overall picture of patterns of sensitivity, these may not reflect local situations because of geographical variations in sensitivity. This report presents the results of three annual surveys of strains isolated in the East End of London and compares them with previous reports from the London area.

Materials and methods

The strains studied were isolated from patients attending the Whitechapel Clinic at the London Hospital before any treatment was given. Cultures were made on Oxoid brain heart infusion agar with 10% horse blood containing 3.0 µg/ml vancomycin and 7.5 µg/ml colistin. Organisms were identified as *N. gonorrhoeae* on the basis of their colonial and microscopical morphology, a positive oxidase reaction, and the production of acid from glucose but not from maltose, lactose, or saccharose. Three surveys were made: June to December 1976 (454 strains), January to March 1977 (225 strains), and May to September 1978 (274 strains).

Address for reprints: Dr A. E. Wilkinson, VD Reference Laboratory, London Hospital, Ashfield Street, London E1 2BL

Received for publication 26 January 1979

SENSITIVITY TESTS

A plate-dilution method was used in which doubling dilutions of antibiotics were incorporated in brain heart infusion agar with 10% horse blood. Oxoid diagnostic sensitivity test agar with 5% lysed horse blood and 1% Kellogg's defined supplement was used in tests for sensitivity to co-trimoxazole.

Growth from an overnight culture was suspended by a vortex mixer in Oxoid nutrient broth to give an opacity corresponding to a McFarland No. 2 tube and transferred to the series of antibiotic-containing plates and an antibiotic-free control by a multipoint inoculator (Denley Instruments, Billingshurst, Sussex). This gave an inoculum of approximately 10^4 colony-forming units (cfu). Control strains of known sensitivity (IRC 3, IRC 5, obtained from the State Serum Institute, Copenhagen) and the Oxford staphylococcus were included in each set of tests. Plates were incubated for 48 hours in candle-extinction jars at 36°C and the MIC taken as the highest dilution of antibiotic which gave no growth or only a single colony.

Results

The results of three surveys from 1976 to 1978 of the sensitivity of gonococci to the six antibiotics tested are shown in Tables 1-3 and are compared with earlier surveys from the London area in Table 4. The patterns of sensitivity to tetracycline and streptomycin among the strains which were found to be insensitive to penicillin are shown in Table 5.

Discussion

PENICILLIN

In the surveys reported by Rodin and Seth (1972) and Taylor and Seth (1975) the method used was the same as in the present study except that a multipoint inoculator was not used, so that the results are comparable. Differences in the medium, technique, and inoculum size used in the other series (Table 4) allow only a general comparison of results. These show a similar trend to that found in the United States and in Denmark, a rise in the proportion of the most resistant strains (MIC 0.125 µg/ml) from 1958 to a peak between 1968 and 1970, followed by a decline until 1976, and a slight increase from this year to 1978. The proportion of strains with an intermediate level of sensitivity (0.03-0.06 µg/ml) also increased from 19.1% in 1976 to 25.3% and 25.6% in 1977 and 1978 respectively. These may be chance variations and further surveys will be necessary to determine whether the proportion of resistant strains is again increasing.

The appearance of β -lactamase-producing strains has not significantly affected the position in the area

Table 1 Sensitivity of gonococci to penicillin, ampicillin, tetracycline, and spectinomycin

MIC (µg/ml)	% of strains		
	1976	1977	1978
Penicillin			
<0.004	30.6	18.2	18.3
0.008	25.6	27.1	27.0
0.015	6.4	9.8	7.3
0.03	7.9	8.4	8.4
0.06	11.2	16.9	17.2
0.125	8.6	10.2	8.0
0.25	5.7	7.1	7.3
>0.5	4.0	2.2	6.6
No. of strains tested	454	225	274
Ampicillin			
<0.004	0.4	0.4	
0.008	19.2	12.4	
0.015	25.0	27.1	
0.03	11.6	14.7	
0.06	12.5	13.3	
0.125	20.1	20.0	
0.25	8.9	11.1	
>0.5	2.2	0.9	
No. of strains tested	224	225	
Tetracycline			
<0.06	1.1	1.8	3.3
0.125	24.7	22.2	38.0
0.25	40.1	40.0	24.1
0.5	21.6	23.1	22.0
1.0	11.5	12.0	12.0
>2.0	1.1	0.9	0.7
No. of strains tested	454	225	274
Spectinomycin			
<5	13.7	13.3	16.1
10	80.4	83.1	71.9
20	5.9	3.6	12.0
40	0	0	0
No. of strains tested	454	225	274

Table 2 Sensitivity of gonococci to streptomycin

MIC (µg/ml)		% of strains		
10	500	1976	1977	1978
S	S	76.7	83.1	90.9
R	S	5.5	1.8	0.7
R	R	17.8	15.1	8.4
No. of strains tested		454	225	274

S = sensitive; R = resistant

Table 3 Sensitivity of gonococci to trimethoprim-sulphamethoxazole (1978)

MIC (µg/ml)		% of strains
TMP	SMX	
0.06	1.87	2.8
0.125	2.375	7.9
0.25	4.75	23.3
0.5	9.5	36.0
1.0	19	23.7
2.0	38	5.9
4.0	76	0.4
No. of strains tested		253

TMP = trimethoprim; SMX = sulphamethoxazole

Table 4 Sensitivity of gonococci to penicillin in the London area (1958-78)

Authors	Year	No. of strains tested	% resistant*
Curtis and Wilkinson	1958	302	14.2
Cradock-Watson <i>et al.</i>	1958	200	10.5
Nicol <i>et al.</i>	1968	91	37.4
Leigh <i>et al.</i>	1969	189	20.0
Phillips <i>et al.</i>	1970	96	35.0
Rodin and Seth	1972	228	32.9
Taylor and Seth	1975	277	30.0
Phillips <i>et al.</i>	1976	264	30.0
Platt	1976	95	25.3
Present series	1976	454	18.3
	1977	225	19.5
	1978	274	21.9

*MIC ≥ 0.125 $\mu\text{g/ml}$ or ≥ 0.2 units/ml.

Table 5 Percentage of resistance to tetracycline or streptomycin or both among gonococci with diminished sensitivity to penicillin (1976-78)

Antibiotic		% of strains		
		1976	1977	1978
Penicillin	R	16.9	36.4	48.3
Tetracycline	S			
Streptomycin	S			
Penicillin	R	9.6	6.8	23.3
Tetracycline	R			
Streptomycin	S			
Penicillin	R	44.6	38.6	10.0
Tetracycline	S			
Streptomycin	R			
Penicillin	R	28.9	18.2	18.3
Tetracycline	R			
Streptomycin	R			
No. of strains		83	44	60

Penicillin R = MIC ≥ 0.125 $\mu\text{g/ml}$; tetracycline R = MIC ≥ 1 $\mu\text{g/ml}$; streptomycin R = MIC ≥ 10 $\mu\text{g/ml}$

served by the Whitechapel Clinic. Three such strains were found in 1976, two in 1977, and two in 1978; they formed less than 0.2% of the gonococci isolated from clinic patients over the period and were traceable to importations from outside the London area.

AMPICILLIN

The distribution of sensitivity of gonococci to ampicillin was similar in 1976 and 1977; 31.2% and 32% of the strains had MICs of ≥ 0.125 $\mu\text{g/ml}$ respectively.

TETRACYCLINE

No changes in the proportion of insensitive strains (MIC ≥ 1.0 $\mu\text{g/ml}$) were detected during the three surveys: 12.6% in 1976, 12.9% in 1977, and 12.7% in 1978. These insensitive strains tended to show decreased sensitivity to penicillin. In 1978 25 of 35 strains with an MIC to tetracycline of ≥ 1.0 $\mu\text{g/ml}$ had MICs of ≥ 0.125 μg penicillin/ml. The

proportion of strains with decreased sensitivity to tetracycline is considerably less than the figure of 30% reported by Phillips *et al.* (1970).

SPECTINOMYCIN

No strains resistant to spectinomycin were found during the period surveyed, but the proportion of strains requiring a concentration of 20 $\mu\text{g/ml}$ for inhibition rose slightly to 12% in 1978. Two of these 33 strains were also insensitive to penicillin, four to tetracycline, and one to streptomycin. Because of the value of spectinomycin for the treatment of infections with β -lactamase-producing strains of gonococci, restriction in its use has been recommended to lessen the risk of emergence of spectinomycin-resistant strains. These show high-level resistance which has been observed to develop during treatment (Reyn *et al.*, 1973). This is evidently a very rare event; Karney *et al.* (1977) found no evidence of increased resistance when this antibiotic was used as a routine form of treatment for 2938 patients over a period of two years. However, while there are equally effective and cheaper alternatives available for routine treatment, restriction of the use of spectinomycin seems a wise precaution.

STREPTOMYCIN

The rapid development of high-level resistance to streptomycin led to its abandonment for the treatment of gonorrhoea. Resistance was usually linked to diminished sensitivity to penicillin. In 1970 about a third of the strains had an MIC of ≥ 12.5 $\mu\text{g/ml}$ (Phillips *et al.* 1970). The results of the present surveys (Table 2) show that the proportion of streptomycin-resistant strains fell progressively, and in 1978 90.9% of the strains were sensitive to a concentration of 10 $\mu\text{g/ml}$. In this period 43 of 60 strains with an MIC of ≥ 1.125 μg penicillin/ml were sensitive to 10 $\mu\text{g/ml}$ of streptomycin. A similar fall in resistance to streptomycin has been reported in surveys in Denmark (WHO, 1978).

CO-TRIMOXAZOLE

Co-trimoxazole was tested only in 1978 (Table 3). There was some correlation between the sensitivity to penicillin and to the trimethoprim-sulphamethoxazole mixture. Thirty-four (60.7%) of 56 strains with an MIC of ≥ 0.125 μg penicillin/ml required ≥ 1.0 $\mu\text{g/ml}$ trimethoprim plus 19 $\mu\text{g/ml}$ sulphamethoxazole for inhibition compared with 21.3% of 197 strains which were sensitive to ≤ 0.06 μg penicillin/ml.

MULTIPLE RESISTANCE AMONG PENICILLIN-INSENSITIVE STRAINS

Gonococci with diminished sensitivity to penicillin often show resistance to other antibiotics such as the

tetracyclines and streptomycin (Table 5). During the three years covered by the surveys there was a progressive decrease in the proportion of strains which had a multiple resistance, and in 1978 almost half the strains which were insensitive to penicillin were sensitive to both tetracycline and streptomycin.

Gonorrhoea is usually treated on the finding of presumptive gonococci in Gram-stained smears before the results of cultures and sensitivity tests are available. This presupposes a knowledge of the usual patterns of sensitivity of the gonococcal strains in local circulation. The results of these surveys show that, with the exception of the rare β -lactamase-producing strains, treatment with penicillin or ampicillin plus probenecid should still give high cure rates in this area. Tetracycline resistance is not common and does not seem to be increasing. Spectinomycin should also be very effective, but its general use is thought to be inadvisable. Further surveys will be needed to see whether the present favourable trend continues.

We wish to thank the World Health Organisation for financial support.

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Failure of iron to promote attachment of gonococci to human spermatozoa under physiological conditions

ALAN P. JOHNSON AND MARY F. OSBORN

From the Division of Communicable Diseases, MRC Clinical Research Centre, Harrow, Middlesex

SUMMARY The effect of iron on the attachment of gonococci to human spermatozoa was investigated using the three iron salts, ferric chloride, ferric nitrate, and ammonium ferric citrate (AFC). Ferric chloride and ferric nitrate were found to be unsuitable for such studies because they were insoluble at physiological pH values, produced a marked decrease in the pH of unbuffered medium (Ringer's solution), and agglutinated spermatozoa. AFC, in contrast, was soluble at physiological pH, did not affect the pH value of Ringer's solution, and did not agglutinate spermatozoa. When gonococci and spermatozoa were mixed together in media with and without AFC, the proportion of spermatozoa with adherent gonococci was approximately the same in each case. Thus, in contrast to a previous report, we have found that the addition of iron does not increase the attachment of gonococci to human spermatozoa.

Introduction

The ability of bacteria to acquire iron from host tissues is an essential feature of the pathogenesis of many bacterial infections (Weinberg, 1978), and recent studies with *Neisseria gonorrhoeae* suggest that this phenomenon may prove to be important in gonorrhoea. Addition of exogenous iron has been shown to promote the growth of gonococci *in vitro* (Kellogg *et al.*, 1968), to induce reversion of type 4 colonies which are unassociated with virulence to colonies with virulence-associated type 1 morphology (Hafiz *et al.*, 1977), to enhance the survival of serum-susceptible strains of *N. gonorrhoeae* in the presence of serum (Johnson *et al.*, 1978; Norrod and Williams, 1978), and to enhance the virulence of gonococci for chicken embryos (Payne and Finkelstein, 1975). Furthermore, strains of gonococci isolated from patients with disseminated disease exhibit an enhanced ability to acquire iron in experimental infections of chicken embryos (Payne, *et al.*, 1978), and one such strain produces an iron-chelating factor which promotes growth of gonococci in low-iron medium (Payne and Finkelstein, 1978).

It has also been reported that addition of iron salts

promotes the attachment of gonococci to human spermatozoa (James *et al.*, 1976). Such an effect could be important because factors which enhance attachment of gonococci to host tissues would tend to increase the likelihood of the organisms successfully colonising host epithelial surfaces. This study was therefore undertaken in an attempt to confirm and extend the observation that iron salts promote attachment of gonococci to spermatozoa.

Materials and methods

BACTERIA

Type 1 gonococci (Kellogg *et al.*, 1963) of strain 192A (McGee *et al.*, 1976) were used in all studies.

REAGENTS

Stock solutions of ferric chloride and ferric nitrate (100 mmol) were prepared, sterilised, and stored as previously described (James *et al.*, 1976). Ammonium ferric citrate (AFC) containing 20.5-22.5% (w/w) iron (Hopkin and Williams) and tri-ammonium citrate (Hopkin and Williams) were each dissolved in distilled water and sterilised by filtration through a 0.22 μ Millipore filter. As the exact amount of iron in the AFC was unknown, stock solutions were prepared on the basis that there was 20.5% (w/w) iron present. However, the actual iron concentration may have been up to 10% more than the values quoted in the results. Ringer's

Address for reprints: Dr A. P. Johnson, Division of Communicable Diseases, MRC Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ

Received for publication 5 January 1979

solution (RS) was prepared as described previously (James *et al.*, 1976), filter-sterilised and stored in plastic containers. Eagle's minimal essential medium (MEM) was prepared by diluting a 10× concentrated solution (GIBCO) to single strength with sterile pyrogen-free water for injection. The MEM was supplemented with HEPES buffer (50 mmol) and the pH was adjusted to a value of 7.2 with sterile 1 mol NaOH.

PREPARATION OF SPERMATOOZOA

Samples of human semen from a single donor were used throughout these studies. The semen samples were diluted to 20 ml in either RS or MEM within five minutes of collection and centrifuged at $1000 \times g$ for five minutes at room temperature. The pelleted spermatozoa were resuspended in fresh medium and washed twice. The spermatozoa were finally resuspended in 5 ml of medium, counted in a haemocytometer, and diluted so that the final concentration was in the range 10^6 – 10^7 cells/ml.

PREPARATION OF ERYTHROCYTES

Five millilitres of blood from each of three donors (blood groups A, AB, and O) were collected separately into heparinised tubes (Sigma). Each sample was diluted to a volume of 25 ml in RS, and the erythrocytes were sedimented by centrifugation at $1000 \times g$ for five minutes. The erythrocytes were resuspended in RS, washed twice, and then resuspended in 25 ml of RS for use in agglutination studies.

MIXTURE OF SPERMATOOZOA AND BACTERIA

Gonococci were grown for 18–22 hours at 37°C on GC agar base (Difco) supplemented with 2% IsovitaleX (Baltimore Biological Laboratories) in an atmosphere of 5% CO₂ in air. The bacteria were scraped from the surface of the agar, suspended in 5–10 ml of either RS or MEM, and agitated on a Rotamix Deluxe at maximal amplitude for 30–60 seconds. The suspension was then centrifuged at

$70 \times g$ for three minutes to remove clumped bacteria. The upper portion of the suspension was removed for use in experiments, care being taken not to disturb the sediment, and the number of viable gonococci was determined by plating out serial 10-fold dilutions. The number of gonococci used in different experiments varied in the range 10^6 – 10^7 colony-forming units/ml.

The test mixture used in attachment experiments consisted of 0.5 ml of gonococcal suspension, 0.1 ml of either an iron solution or control fluid (that is distilled water or a solution of ammonium citrate), and 0.4 ml of sperm suspension. In any given experiment, the spermatozoa and gonococci were both suspended in either RS or MEM but never in a mixture of the two. The iron solutions were routinely preincubated with the gonococci for 20–30 minutes and then, following the addition of spermatozoa, the test mixtures were incubated in a water bath at 37°C for 15 minutes, during which time the suspensions were agitated at intervals by gentle manual shaking.

EVALUATION OF ATTACHMENT OF GONOCOCCI TO SPERMATOOZOA

At the end of the incubation period, two separate drops from each suspension were placed on a glass slide using a Pasteur pipette and allowed to dry in air. The slides were fixed overnight in methanol, then Gram stained. A minimum of 200 spermatozoa for each drop was counted at $\times 650$ magnification, and the percentage with one or more diplococci attached was determined. All slides were coded before microscopical examination to reduce observer bias in determining the degree of attachment.

Results

SOLUBILITY OF IRON SALTS

In initial experiments, attempts were made to supplement HEPES-buffered MEM, pH 7.2, with iron in the form of either ferric chloride or ferric nitrate. Both these salts however reacted upon mixing

Table 1 *Effect of iron compounds and pH value on attachment of gonococci to spermatozoa*

Suspension containing	Suspending medium	pH value	Agglutination of spermatozoa	% of spermatozoa (mean $\pm$ SD) with adherent gonococci
Gonococci + spermatozoa	RS	6.8	—	28.8 $\pm$ 8.4
	RS + 0.1 mmol FeCl ₃ .6H ₂ O	3.1	+	74.4 $\pm$ 13.4*
	RS + 0.1 mmol Fe(NO ₃) ₃ .9H ₂ O	3.1	+	76.4 $\pm$ 5.2*
	RS	3.1	—	45.7 $\pm$ 8.5
	RS + AFC	6.4	—	32.5 $\pm$ 5.7
	RS + AC	6.8	—	35.9 $\pm$ 5.3
Spermatozoa	RS	6.8	—	
	RS + 0.1 mmol FeCl ₃ .6H ₂ O	3.1	+	

+ Positive — negative

* Counts performed on unclumped spermatozoa only

AFC = ammonium ferric citrate (final concentration of iron approximately 10 µg/ml); AC = ammonium citrate (final concentration 40 µg/ml); RS = Ringer's solution

with MEM to produce insoluble orange precipitates. In subsequent experiments therefore RS was used as the diluent, since ferric chloride and ferric nitrate are soluble in this fluid (James *et al.*, 1976). RS is a simple salt solution with negligible buffering capacity, and several batches tested during the course of these studies had pH values within the range 6.2-6.8. With all batches of RS studied, addition of either ferric chloride or ferric nitrate at a final concentration of 0.1 mmol resulted in a dramatic drop in the pH value to within the range 3.0-3.5 (Table 1). Attempts to adjust the pH of the solution to its original value by addition of sodium hydroxide resulted in the formation of a precipitate. To study the effect of iron on gonococcal attachment to cells at physiological pH values, a further group of experiments was therefore conducted in which the soluble iron-complex AFC was used. When added to RS, AFC (final concentration of iron approximately 10 µg/ml) lowered the pH value only marginally (Table 1) and had no effect at all on the pH of MEM buffered with HEPES (Table 2).

AGGLUTINATION OF CELLS BY IRON SALTS

To assess the effect of iron on the interaction of gonococci and spermatozoa, attachment studies were

Table 2 *Effect of ammonium ferric citrate on attachment of gonococci to spermatozoa*

<i>Suspending medium</i>	<i>pH value</i>	<i>% of spermatozoa (mean ± SD) with adherent gonococci</i>
MEM	7.2	35.7 ± 1.5
MEM + AFC	7.1	33.1 ± 2.8
MEM + AC	7.2	32.1 ± 0.6

AFC = ammonium ferric citrate (final concentration of iron approximately 10 µg/ml); AC = ammonium citrate (final concentration 40 µg/ml)

performed using gonococci and spermatozoa suspended in either RS or RS supplemented with ferric chloride, ferric nitrate, or AFC. In addition, gonococci and spermatozoa were suspended in RS, the pH of which had been adjusted (by addition of HCl) to a value comparable to that of RS supplemented with ferric chloride or ferric nitrate (Table 1). When a sperm suspension was added to RS supplemented with either ferric chloride or ferric nitrate (final pH 3.1) a white precipitate was produced which was seen on microscopical examination to consist of agglutinated spermatozoa (Fig. 1). Gonococci were not necessary for agglutination since it readily occurred in suspensions containing only

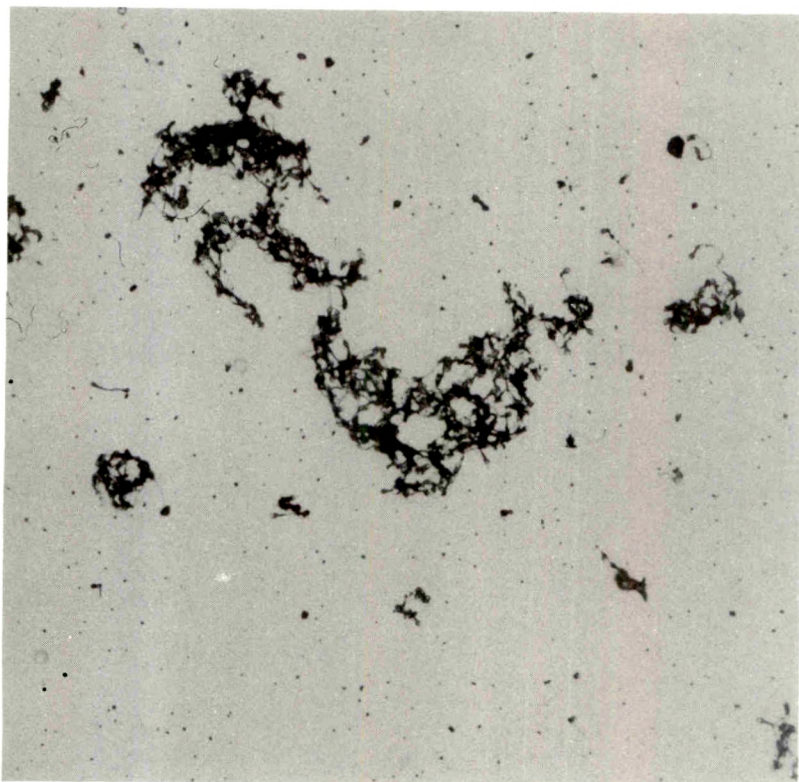


Fig. 1 *Agglutination of human spermatozoa suspended in Ringer's solution containing 0.1 mmol ferric chloride. (Gram stain; × 180 magnification)*

spermatozoa (Table 1). Agglutination was not detected however when spermatozoa were added to RS not supplemented with iron (Fig. 2), to RS at a low pH value, or to RS supplemented with AFC (Table 1). Similarly in experiments in which AFC or ammonium citrate was added to MEM buffered with HEPES (pH 7.2) no agglutination of spermatozoa occurred. The agglutination of cells in the presence of ferric chloride or ferric nitrate was not specific for spermatozoa, since an identical pattern of agglutination resulted when erythrocytes rather than sperm were added to the various media described above.

EFFECT OF IRON SALTS ON ATTACHMENT OF GONOCOCCI TO SPERMATOZOA

The proportion of spermatozoa with gonococci attached to them did not vary markedly when cells suspended in RS pH 6.8 were compared with cells suspended in RS containing either AFC or ammonium citrate (Table 1). A slight increase in the proportion with adherent gonococci was however observed with spermatozoa suspended in RS pH 3.1 (Table 1). When spermatozoa were suspended in RS supplemented with either ferric chloride or ferric nitrate, accurate determination of the proportion with attached gonococci was not possible owing to

the tendency of the spermatozoa to agglutinate. In such samples however counts performed on free spermatozoa indicated that about 75% of the cells had adherent gonococci (Table 1).

In experiments in which HEPES-buffered MEM (pH 7.2) was used as the suspending medium, addition of iron (at a final concentration of 10 $\mu\text{g/ml}$) in the form of AFC did not have any effect on the proportion of spermatozoa to which gonococci attached (Table 2).

Discussion

Previous work on the interaction of gonococci with spermatozoa *in vitro* indicated that adherence of the bacteria is enhanced in the presence of iron salts (James *et al.*, 1976). The findings presented here however suggest that the circumstances of their study may have been rather adverse and that the degree of attachment may have been raised for a number of reasons. In our study, addition of the iron salts, ferric chloride or ferric nitrate, produced immediate agglutination of the sperm cells. Some cells entangled in the clumps would be less accessible to gonococci and consequently the ratio of gonococci to accessible

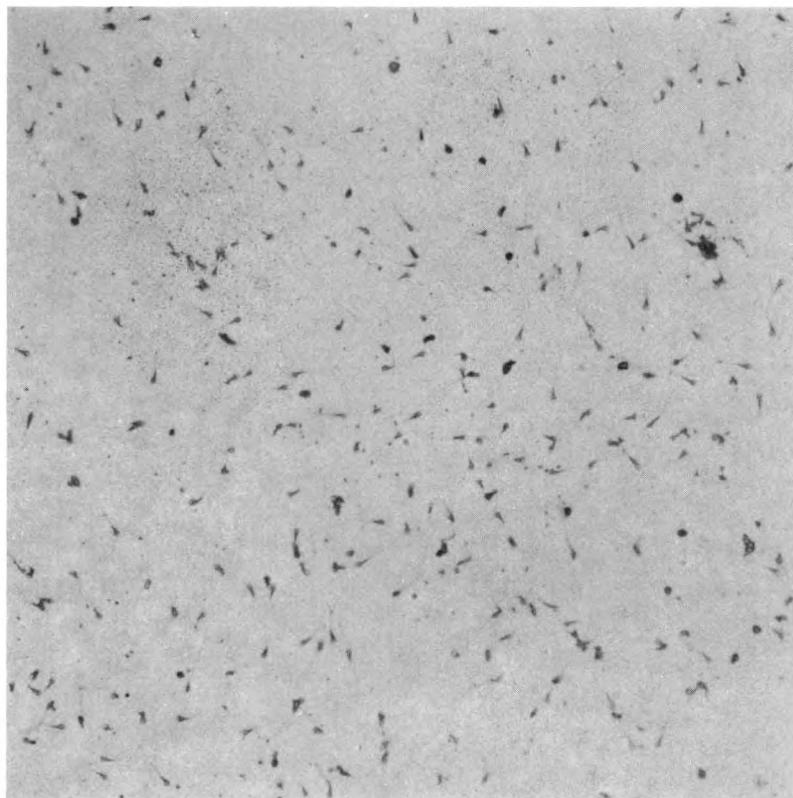


Fig. 2 Human spermatozoa suspended in Ringer's solution not supplemented with iron salts. (Gram stain; $\times 180$ magnification)

spermatozoa (that is, spermatozoa which have an opportunity to come into contact with a gonococcus) would be increased. This would be expected to result in an apparent increase in the degree of attachment of gonococci if counts were performed only on free spermatozoa, since the degree of attachment has been shown to be directly related to the ratio of gonococci to spermatozoa in the reaction mixture (James-Holmquest *et al.*, 1974). In our study, the agglutination of spermatozoa by the iron salts appeared to be non-specific with regard to cell type, since erythrocytes of three different blood groups were also agglutinated. James *et al.* (1976), in contrast, made no comment about agglutination of spermatozoa; however they used pooled semen samples which had been frozen in glycerol and incubated their mixtures on a continually shaking water bath. These experimental conditions were slightly different from ours and may possibly have led to less agglutination of spermatozoa than seen by us.

Attachment of gonococci to spermatozoa in the presence of ferric chloride or ferric nitrate was also apparently enhanced because these iron salts produced a marked decrease in the pH value of the unbuffered suspending medium (RS). When the attachment of gonococci to spermatozoa suspended in RS pH 6.8 was compared to the attachment observed with gonococci and spermatozoa suspended in RS pH 3.1 (the pH value of RS supplemented with 0.1 mmol ferric chloride or ferric nitrate) enhanced attachment occurred at the lower pH value (Table 1). Thus, some of the enhanced attachment apparently produced by ferric chloride or ferric nitrate is due, at least in part, to the effect these salts have on the pH of the medium. This is further borne out by the observation that iron in the form of AFC, which failed to lower the pH value of RS to any marked degree, failed to enhance attachment of the bacteria to the spermatozoa.

The observation that the pH value of RS supplemented with ferric chloride or ferric nitrate at a final concentration of 0.1 mmol is in the range 3.0-3.5 has important implications in that this pH range is several orders of magnitude lower than that found *in vivo*. Thus, observations made on the interaction of gonococci with spermatozoa under these conditions may not be relevant to interactions that occur under conditions of physiological pH. Since ferric chloride and ferric nitrate were insoluble

at pH values close to neutrality, subsequent studies were performed using the soluble iron complex AFC. This compound was soluble in both RS pH 6.8 and in MEM buffered with HEPES at pH 7.2, the normal pH value of spermatozoa (Diem and Lentner, 1970). The presence of AFC did not enhance attachment of gonococci to spermatozoa with either RS or MEM as the suspending medium. Thus, no evidence was obtained that iron enhanced attachment of gonococci to sperm cells under conditions of physiological pH.

We would like to thank Dr David Taylor-Robinson for his advice and criticism regarding the manuscript and Mr John Clark for his help with the photography.

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Frequency of simultaneous carriage of *Neisseria gonorrhoeae* and *Neisseria meningitidis*

K. ØDEGAARD AND T. W. GEDDE-DAHL

From the Departments of Bacteriology and Epidemiology, National Institute of Public Health, Oslo, Norway

SUMMARY Gonococci were found by culture in 112 of 442 patients suspected of having gonorrhoea. Meningococci were cultured from the pharynx more than twice as often in patients harbouring gonococci (26%) than in patients from whom gonococci could not be cultured (11%). These results could indicate an individual susceptibility to *Neisseria* organisms or they could be caused by a difference in the behaviour of patients with and without gonorrhoea, the conduct of the former making them not only more likely to be infected with *Neisseria gonorrhoeae* but possibly more exposed to *Neisseria meningitidis* as well.

Introduction

Willcox *et al.* (1977) found that of 150 pharyngeal cultures from patients who were examined because they had a history of oral sexual intercourse *Neisseria meningitidis* was isolated nearly six times more often in patients with genital gonorrhoea than in those whose genital cultures gave negative results. On the other hand, *Neisseria gonorrhoeae* was found 2½ times more often in those who carried the meningococcus in the pharynx than in those who did not. Willcox *et al.* suggest that if these findings could be confirmed they could indicate an individual susceptibility to the acquisition of *Neisseria* organisms that would merit further investigation.

Rufli (1978), in a series of 2412 patients, isolated *N. meningitidis* 2½ times more often from patients with genital gonorrhoea than from patients without and genital *N. gonorrhoeae* almost twice as often from patients with tonsillar *N. meningitidis* than from patients without. The aim of the present paper is to see if these findings could be confirmed.

Patients and methods

Unselected patients suspected of having gonorrhoea who visited the Department of Venereal Diseases at the Oslo Board of Health from the middle of September to the end of October 1978 were included

in the study. On their first visit, samples were taken from the urethra, cervix, rectum, and pharynx in women and from the urethra and pharynx in men. In a few cases rectal samples were also taken from men.

The samples were taken with charcoal-impregnated swabs, which were placed in solid Stuart's transport medium, and sent to the laboratory, where they were cultured from two to five hours later. The specimens were streaked on selective chocolate agar medium containing colistin, lincomycin, trimethoprim, and nystatin (Ødegaard, 1971; Ødegaard *et al.*, 1975) and incubated at 37°C in an atmosphere containing 5% CO₂. The gonococci and the meningococci were identified on the basis of colonial and microscopical examination, oxidase and fermentation reactions, as well as growth requirements. Glucose, maltose, lactose, and sucrose were used for fermentation tests. Nutrient agar incubated at 22°C and Dubos oleic acid agar medium incubated at 36°C in an atmosphere containing 5% CO₂ were used for growth requirements.

The meningococci were distinguished from the gonococci by their ability to ferment maltose and to grow on Dubos oleic acid agar medium. Both organisms failed to ferment sucrose or lactose or to grow on nutrient agar at 22°C.

Results

Samples from 442 patients (149 women and 293 men) were collected. *N. gonorrhoeae* was isolated by culture in 112 (25%) patients (42 women and 70 men), while *N. meningitidis* was isolated in 64 (14%)

Address for reprints: Dr K. Ødegaard, National Institute of Public Health, Geitmyrsveien 75, Oslo 1, Norway

Received for publication 7 February 1979

patients (22 women and 42 men). In four patients *N. gonorrhoeae* was cultured from the pharynx. In none of the cases was *N. meningitidis* cultured from the genital or rectal samples.

In 330 patients with negative culture results for *N. gonorrhoeae*, *N. meningitidis* was cultured from 35 (11%) (12 women and 23 men), whereas in the 112 patients with positive culture results for *N. gonorrhoeae*, *N. meningitidis* was cultured from 29 (26%) (10 women and 19 men). In 378 patients with negative culture results for *N. meningitidis*, gonococci were found in 83 (22%) (32 women and 51 men) whereas in the 64 patients from whom *N. meningitidis* was cultured, gonococci were found in 29 (45%) (10 women and 19 men). The results are shown in the Table.

Thus, a highly significant correlation appears to exist between the occurrence of gonococci and meningococci.

The relative risk of harbouring meningococci when gonococci are found is 2.44 with an approximate 95% confidence interval of 1.4-4.2.

Table Culture results for *Neisseria gonorrhoeae* and *Neisseria meningitidis* in 442 patients

	<i>Culture results</i>		
	<i>N. meningitidis</i>		
<i>Culture results</i>	<i>Positive</i>	<i>Negative</i>	<i>Total</i>
<i>N. gonorrhoeae</i>			
Positive	29	83	112
Negative	35	295	330
Total	64	378	442

$$\chi^2 = 14.6 \text{ } P < 0.001$$

Discussion

The highly significant correlation between the occurrence of gonococci and meningococci is in agreement with the findings of Willcox *et al.* (1977) and Ruffi (1978).

The results could indicate either an individual susceptibility to the acquisition of *Neisseria* organisms or a difference in behaviour of patients with and without gonorrhoea, the conduct of the former making them not only more likely to be infected with *N. gonorrhoeae* but possibly more exposed to the acquisition of *N. meningitidis* as well. Both possibilities might also co-exist.

The findings of Lee *et al.* (1978) of recurrent bacteraemic infections due to *Neisseria* associated with familial deficiency of the seventh component of complement indicate, however, that individual susceptibility factors may play a part.

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Meningococcal colonisation misdiagnosed as gonococcal pharyngeal infection

ROBERT C. NOBLE AND ROBERT M. COOPER*

From the Division of Infectious Diseases, Department of Medicine, University of Kentucky College of Medicine, Lexington, Kentucky, USA

SUMMARY Three cases of pharyngeal gonorrhoea were misdiagnosed in patients harbouring meningococci which failed to utilise maltose in CTA medium. The microbiological identity of the isolates was established by other means. Although meningococci giving atypical reactions to carbohydrate utilisation tests occur only rarely, the possibility of a misdiagnosis of gonorrhoea, and its social and legal repercussions, should be considered.

Introduction

Meningococci and gonococci usually infect the host at specific and different sites. This predilection for certain areas of the host, however, is not always present. Contrary to the usual experience, meningococci may infect urogenital and anal sites and gonococci may infect the pharynx and central nervous system (Holmes *et al.*, 1971; Wiesner *et al.*, 1973; Faur *et al.*, 1975a; Noble *et al.*, 1979). Pharyngeal infection by *Neisseria gonorrhoeae* is now recognised as another manifestation of gonococcal disease and these infections are more common in homosexual men, pregnant women, and other individuals practising oral genital sexual contact (Wiesner *et al.*, 1973; Courman *et al.* 1974). Meningococci may also be found in the pharyngeal cultures of healthy individuals and patients attending venereal disease clinics (Weisner *et al.*, 1973; Noble *et al.*, 1979). Carbohydrate utilisation reactions are necessary to distinguish meningococci from gonococci since both organisms may be present in the pharynx. Unfortunately, these reactions are not always reliable and confusion between the two pathogens can occur. The following case reports show that a misdiagnosis of pharyngeal gonorrhoea is possible in patients who harbour meningococci with atypical carbohydrate utilisation reactions.

Case reports

CASE 1

A 21-year-old, white, married woman was admitted to the University of Kentucky Medical Center because of increasing dyspnoea. During the previous two weeks she had been exposed to a sister who had varicella. Six days before her admission the patient developed attacks of shivering and fever accompanied by vesicular lesions, which appeared initially on her chest. Three days before admission, she began to have a dry, hacking cough and shortness of breath.

On admission, she was dyspnoeic with a florid vesiculopustular rash covering her entire body. She had a fever of 40°C; her respiration rate was 60 per minute, her blood pressure 110/60 mm Hg, and her pulse rate regular at 110 per minute. The laboratory values indicated hypoxia, a normal white blood cell count with a predominance of stab cells, and a low platelet count. The chest radiograph showed diffuse fluffy infiltrates affecting all lung fields. A pharyngeal culture, performed because she complained of a sore throat, initially gave a positive result for gonococci. A cervical culture gave a negative result for gonococci. Following penicillin therapy, a repeat pharyngeal culture result for gonococci was negative. She made an uneventful recovery. At the time of her illness, she had been living at home with her parents because her husband had been in prison for one year. She denied any extramarital sexual contacts.

CASE 2

A 19-year-old, white, single man was seen in the Lexington-Fayette County Venereal Disease Clinic complaining of an urethral discharge, dysuria of three days' duration, and a sore throat. He had a thin, mucoid, urethral discharge and cervical lymph nodes which were enlarged bilaterally. His tonsils were enlarged, but there was no exudate. He denied orogenital sexual contact. Microscopical examination of the urethral exudate showed numerous polymorphonuclear

*Present affiliation: Department of Internal Medicine, Lexington Clinic, Lexington, Kentucky

Address for reprints: Dr Robert C. Noble, Division of Infectious Diseases, Department of Medicine, University of Kentucky College of Medicine, Lexington, Kentucky 40536, USA

Received for publication 7 February 1979

leucocytes but no bacteria. Culture of the urethral exudate showed no growth. His pharyngeal culture result was initially reported as positive for gonococci. He was treated with oral tetracycline and a follow-up pharyngeal culture result was negative.

CASE 3

A 20-year-old, white, married man attended the Lexington-Fayette County Venereal Disease Clinic complaining of a painful urethral discharge of three days' duration. He did not have a sore throat. Clinical examination was unremarkable except for the presence of a purulent urethral exudate. Microscopical examination of the exudate showed numerous polymorphonuclear leucocytes and many Gram-negative intracellular diplococci. A urethral culture showed the presence of oxidase-positive, Gram-negative diplococci. Unfortunately, this culture was lost and could not be further identified. A pharyngeal culture result was initially reported as positive for gonococci. He was treated with oral ampicillin and probenecid but failed to return for a follow-up culture.

Materials and methods

MICROBIOLOGICAL METHODS

The pharyngeal cultures were taken with cotton-tipped swabs, inoculated on to modified Thayer-Martin medium containing vancomycin, colistin, nystatin, and trimethoprim (Martin and Lester, 1971), and incubated for 24 hours at 36°C in an atmosphere containing 5% CO₂. The isolates were initially identified by bacterial morphology, Gram stain, and oxidase reaction (1% solution of N, N-dimethyl-p-phenylenediamine monohydrochloride). The sugar utilisation reactions were initially performed in CTA medium (Baltimore Biologic Laboratory, Cockeysville, Maryland). The medium contained the test carbohydrates, dextrose, maltose, sucrose, fructose, and lactose, in 1% con-

centrations. In addition, the isolates were identified by the modified rapid fermentation procedure of Kellogg and Turner (Brown, 1974). Fluorescent antibody tests on the isolates were performed with Bacto-FA *N. gonorrhoeae* serum (Difco Laboratories, Detroit, Michigan). Meningococcal serogrouping was performed by the slide agglutination technique with antisera for serogroups A, B, C, D, X, Y, and Z. The isolates were stored at -70°C in tryptic soy broth (Difco) in 13% glycerol.

GONOCOCCAL AUXOTYPING

Auxotyping by the method of Catlin was performed twice on each isolate (Catlin, 1973). Gonococci of a known auxotype were included as controls.

ACRYLAMIDE GEL ELECTROPHORESIS

Gel electrophoresis of the cell proteins of the isolates was performed as previously described (Noble and Schell, 1978). The intact micro-organisms were lyophilised, heated in lauryl sulphate with 2-mercaptoethanol, and the solubilised cell proteins were subjected to alkaline sodium dodecyl sulphate gel electrophoresis. The resulting electrophoresis patterns were scanned with a spectrophotometer.

Results

The results are given in the Table. An acid reaction was present in all tubes containing dextrose when the isolates were tested in CTA medium and by the rapid fermentation procedure. We found no reaction in the tubes containing maltose and CTA medium even though the isolates were repeatedly tested and incubated for several days. The State reference laboratory tested the isolates in tubes containing maltose and CTA medium, however, and found a

Table Results of microbiological tests on isolates from three patients

Determination	Results for isolates from Cases		
	1	2	3
Growth on modified Thayer-Martin medium	+	+	+
Oxidase reaction	+	+	+
Gram-negative diplococcus	+	+	+
Fluorescein-labelled antigonococcal serum	-	-	-
Meningococcal antiserum	Group A	Group Z	Group Z
Carbohydrate utilisation	CTA*/RFP†	CTA/RFP	CTA/RFP
Dextrose	+/+	+/+	+/+
Maltose	-/+	-/+	-/+
Lactose	-/-	-/-	-/-
Fructose	-/-	-/-	-/-
Sucrose	-/-	-/-	-/-
Growth on auxotyping media			
Complete medium	+	+	+
Cystine-deficient medium	+	+	+

+ Positive - negative

*CTA agar

†Rapid fermentation procedure

neutral rather than an acid reaction. Thus both laboratories found atypical reactions in the CTA medium containing maltose. Isolates tested by the rapid fermentation procedure produced an acid reaction in the tubes containing dextrose and maltose.

A negative reaction occurred when the isolates were tested by the fluorescein labelled anti-gonococcal serum. Growth of the isolates on the gonococcal auxotyping media was characteristic of meningococci rather than of gonococci. Gonococci require cystine and meningococci do not. All three isolates were able to grow in the absence of cysteine. The protein electrophoresis patterns of the three isolates differed from those observed with gonococcal isolates.

Discussion

Carbohydrate utilisation tests are generally used to confirm an isolate as either *N. gonorrhoeae* or *Neisseria meningitidis*. These tests are generally performed in a supporting medium and CTA medium is one of the most commonly used for this purpose. In the present study, CTA medium was the only medium used by the routine laboratory for this purpose. CTA medium has, however, been associated with aberrant utilisation of carbohydrates by both gonococcal and meningococcal isolates. The reaction pertinent to this report is that certain meningococci may fail to utilise maltose and are thus mistaken for gonococci (Kingsbury, 1967; Hollis *et al.*, 1970; Faur *et al.*, 1975b). The failure of some meningococci to utilise maltose has been associated with resistance to sulphonamide and is due to the absence of either maltose permease or maltose phosphorylase activity (Kingsbury, 1967).

Although a number of alternative media for the carbohydrate tests have been suggested, none has gained universal acceptance. The rapid fermentation procedure was used successfully in our report, but its use also has problems. Incorrect results may occur if the maltose used in this procedure is not absolutely pure (Kellogg and Turner, 1973; Brown, 1974; Morse and Bartenstein, 1976). Kellogg and Turner (1973) found that only one of four lots of one commercial source of maltose was satisfactory. Morse and Bartenstein (1976) also experienced difficulty with their maltose source and used maltose supplied by Brown (see above). The commercially available fluorescein-labelled antigenococcal serum used in the present study has been shown to contain cross-reactions with meningococcal isolates. These have varied from weak (Blenk *et al.*, 1978) to strong enough to cause difficulty in distinguishing meningococci from gonococci (Hare, 1974). Pollock (1976) found that 5% of nongonococcal *Neisseria*

gave positive results when tested with this reagent. Non-commercial immunofluorescent anti-gonococcal sera are also used to identify gonococci (Lind, 1975). Olcen *et al.* (1978), however, believe that immunofluorescent tests with unabsorbed conjugates cannot be recommended for confirmation of gonococci from extragenital sites and that absorbed conjugates are less suitable for routine use since their reactivity may drop rapidly after one month of storage. Although gonococcal auxotyping may be helpful in distinguishing gonococci from meningococci in problem cultures, it is not practical for routine diagnostic use because the media are difficult to prepare. Patterns of cell proteins on acrylamide gels have not been sufficiently evaluated for diagnostic purposes, and the gel electrophoresis technique used in our study provided evidence only that the isolates were not gonococci.

The appearance of meningococci that fail to utilise maltose is fortunately uncommon; the frequency has ranged from 0.5 to 5.7% of meningococcal isolates from clinical surveys (Hollis *et al.*, 1970; Faur *et al.*, 1975b). This was also our experience. Cases 2 and 3 came to light as a result of pharyngeal cultures taken from 2000 patients attending a venereal disease clinic (Noble *et al.*, 1979). Gonococcal colonisation of the pharynx was present in 1.3% (27/2000) and meningococcal colonisation in 9.5% (189/2000) of the patients.

The physicians of our three patients were unaware that the carbohydrate utilisation reactions might be unreliable. The two isolates from the young men were first suspected of not being gonococci when their protein electrophoresis patterns were examined. The young woman was insistent that her diagnosis was in error, however, since she had been faithful to her husband, who at the time of her illness was in prison. Her story and complaints were initially regarded with scepticism. Although she accepted the reason for the error, the misdiagnosis of a sexually transmitted disease might have been more painful for her and her physician. A misdiagnosis of gonorrhoea may have additional legal and social implications if the patient is a child. Positive gonococcal culture results may be found in children who are victims of sexual abuse. Meningococci also occur occasionally, however, in the pharyngeal cultures of children (Gold *et al.*, 1978), and confusion of these two *neisseriae* is possible. Physicians should be aware that the carbohydrate utilisation reactions, usually considered as the basic criterion of the diagnosis of *Neisseria*, may on occasion be misleading. This knowledge should encourage an open dialogue between the physician and the diagnostic laboratory in an effort to prevent a microbiological misdiagnosis of gonorrhoea.

We thank Philip G. Weiler, Commissioner of Health, Lexington-Fayette County Health Department for his co-operation. We are also grateful to Ms Norma J. Cambron and Dr George E. Kilgore, Microbiology Branch, Department for Human Resources, Commonwealth of Kentucky, for their help with the identification of the meningococci and the fluorescent antibody determinations. We thank Dr W. K. Harrell, Biologic Reagents Section, Center for Disease Control, Atlanta, Georgia, and Dr M. D. Moody, Burroughs Wellcome Company, Research Triangle Park, NC, for their donation of the meningococcal grouping antisera. We appreciate the suggestions of Dr Ernest Stolz, Department of Dermatology, Erasmus University, Rotterdam, The Netherlands. The study was made possible by a grant from Alexandrine and Alexander Sinsheimer Fund.

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Double-blind trial of amoxycillin and ampicillin plus probenecid in the treatment of gonorrhoea in men

A. R. BRATHWAITE

From the Comprehensive Health Centre, Kingston, Jamaica

SUMMARY In a double-blind study amoxycillin 3 g or ampicillin 3 g plus probenecid 1 g were randomly administered as a single oral dose to 160 men with uncomplicated gonococcal urethritis. The cure rate for each drug group was 98.6%. Tolerance to oral medication was good and no evidence of allergy or toxicity to either drug was shown. No case of post-gonococcal urethritis was observed over a 14-day follow-up period. Thus, both drug regimens proved very effective in treating uncomplicated gonococcal urethritis in men.

Introduction

Amoxycillin is an acid-stable, semisynthetic penicillin which is closely related to ampicillin having a similar antibacterial spectrum and level of activity (Sutherland and Rolinson, 1971; Sutherland *et al.*, 1972). When taken orally amoxycillin is better absorbed, and mean peak serum concentrations at two hours are twice those of an equivalent dose of ampicillin (Croydon and Sutherland, 1970). Amoxycillin is not converted into ampicillin in the body.

Several trials using both amoxycillin and ampicillin in the treatment of uncomplicated gonorrhoea have been reported (for example, Karney *et al.*, 1974). No such studies, however, have been reported from the Caribbean area. For this reason a double-blind study was conducted to compare the efficacy of ampicillin 3 g plus probenecid 1 g with amoxycillin 3 g, since the latter had been shown to give the best results.

Patients and methods

Men with uncomplicated gonorrhoea attending the sexually transmitted diseases section of the Comprehensive Health Centre during the period October to December 1977 were admitted to the study. A presumptive diagnosis was made by microscopical identification of typical Gram-

negative intracellular diplococci in smear specimens taken from the urethra. At the same time, material for culture was plated on Thayer-Martin (VCN) media and incubated immediately in candle-extinction jars at 37°C. These were examined for oxidase-positive colonies after 24 and 48 hours' incubation. Gram-stains from typical colonies were examined microscopically.

Patients were then each given a packet of medication containing six 500-mg capsules of either ampicillin plus probenecid or amoxycillin plus placebo tablets to take under direct supervision of a senior member of the clinic staff. The drugs had been coded in random order and prepaced by the manufacturers in identical white capsules, placebo probenecid tablets being used with the amoxycillin. The coding used was unknown to the clinic staff. Patients who were allergic to penicillin, or who were unlikely to attend for follow-up, were excluded from the trial.

Patients were advised to refrain from sexual intercourse and to return to the clinic three, seven, and 14 days after treatment. At each visit, they were questioned about symptoms and sexual contacts and the original instructions were repeated. Gram stains and cultures were also repeated as performed at the first visit.

Treatment was considered to have failed if presumptive gonococci were found in smears and cultures within 14 days in patients who denied further sexual intercourse. Patients were considered to be reinfected if gonococci were found and sexual intercourse admitted to during this follow-up period.

Post-gonococcal urethritis (PGU) was diagnosed if

Address for reprints: Dr A. R. Brathwaite, Comprehensive Health Centre, 55 Slipe Pen Road, Kingston 5, Jamaica, West Indies

Received for publication 17 October 1978

a urethral discharge containing at least 10 leucocytes per field on microscopy using a $\times 100$ objective was present.

Results

Eighty patients were treated with each regimen. Their age range was similar to that of patients with gonorrhoea seen routinely at the clinic (Table 1).

Table 1 *Ages (in years) of the two treatment groups*

Drug regimen	Mean	Median	Mode	Range
Amoxycillin	27.6	24.2	23	16-77
Ampicillin plus probenecid	26.2	24.2	20	16-48

They were all negroes in the lower socioeconomic class. Four patients in the group treated with amoxycillin and two in the group treated with ampicillin and probenecid were married; the remaining patients were single. The mean number of sexual partners in the preceding month was 1.5 and 1.7 in the former and latter groups respectively.

Of those patients treated with ampicillin 3 g plus probenecid 1 g, 71 (98.6%) of 72 patients who were followed up for 14 days were cured. Seven patients were reinfected and one defaulted in this group (Table 2).

Of those patients treated with amoxycillin 3 g, 71 (98.6%) of 72 patients who were followed up for 14 days were cured. Six patients were reinfected and two defaulted (Table 2).

Of the 13 patients with reinfections all had negative results to tests for gonococci on the third day (Table 3). On the seventh day, eight patients had positive results, four results remained negative, and one patient defaulted. By the 14th day, the four patients who had had negative results and the one who had failed to attend previously all had positive culture results. All admitted to re-exposure to infection.

By contrast, both patients in whom treatment failed had positive results to investigations taken on the third day. The results of one patient remained positive on the seventh day when he was removed from the study. The other patient who did not attend

for follow up on the seventh day had a positive result when he attended on the 14th day. Both these patients had minimal symptoms (Table 3).

No case of PGU was identified during the 14-day follow-up period.

Table 3 *Follow-up results in 15 cases of failure/reinfection*

Results	Follow-up period (days)		
	3	7	14
Positive	2	9*	6*
Negative	13	4	0
Removed from study	0	0	9*
Defaulted from visit	0	2*	0
Total	15	15	15

*One treatment failure

Discussion

Although, for technical reasons, it was not possible to perform antibiotic sensitivity tests on gonococcal strains isolated in this study, the cure rate of 98.6% with either regimen is very satisfactory when it is remembered that in 1971 74% of strains isolated at this clinic gave minimum inhibitory concentrations of penicillin of 0.1 unit/ml and over (Fleming *et al.*, 1974). These results are comparable to some obtained from areas with a much lower incidence of less sensitive strains. Alergant (1973) reported a cure rate of 94.2% with amoxycillin 1 g plus probenecid 1 g in Liverpool; Willcox (1974), in London, obtained a cure rate of 98.8% using amoxycillin 3 g in two oral doses five hours apart; Karney *et al.* (1974) had a cure rate of 95% with amoxycillin 3 g and of 98% with ampicillin 3.5 g plus probenecid 1 g in Seattle; Deal *et al.* (1974), in Florida, reported a 97% cure rate with amoxycillin 3 g; Gurwith *et al.* (1974) using amoxycillin 3 g and 2 g plus probenecid 0.5 g obtained cure rates of 95.4% and 89.5% respectively in Manitoba; Roberts (1974) obtained rates of 84% and 95% using amoxycillin 1 g and 3 g respectively in Tennessee; and Wolk (1974), in California, claimed a cure rate of 94% in men using amoxycillin 3 g.

Lassus *et al.* (1975), in Helsinki, had a cure rate of 100% with amoxycillin 3 g if the two possible reinfections are excluded. In London, Price and Fluker (1975) using amoxycillin 3 g obtained a cure

Table 2 *Results of the two treatment regimens in patients followed up for 14 days*

Drug regimen	Cure		Failure		Reinfection		Defaulted		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
Amoxycillin 3g	71	98.6	1	1.4	6		2		80	100
Ampicillin 3g plus probenecid 1g	71	98.6	1	1.4	7		1		80	100

rate of 99% in men and 95% in women assessed at the first follow-up three days later. Thin *et al.* (1977), in London, in the only double-blind study in this series, claimed cure rates of 86% and 94% using amoxycillin 1 g and 3 g respectively (both given with probenecid). In all of these studies cure rates were assessed within 14 days of treatment.

The early (upward) part of the blood concentration curve following administration of amoxycillin 3 g has been shown by Neu (1974) to approximate that following ampicillin 3.5 g plus probenecid 1 g, but disappearance was faster with amoxycillin. Kvale *et al.* (1971) claimed a cure rate of 96% in men treated with ampicillin 3.5 g plus probenecid 1 g for acute gonococcal urethritis in the Philippines, an area with a high incidence of strains insensitive to penicillin. Their result is similar to that found by Karney *et al.* (1974) and to that of the present trial. Whereas Kvale *et al.* (1971) found a 47.9% incidence of PGU during a 21-day follow-up period, however, no cases of PGU with either regimen were seen in this trial at 14 days.

Amoxycillin (Amoxil) was kindly supplied by Beecham Pharmaceuticals Ltd. and probenecid (Benemid) by Merck Sharp and Dohme Ltd. The help of Mr Paul Gordon and Mrs Enid Miller, of the staff of the Comprehensive Health Centre, is gratefully acknowledged.

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Single-dose minocycline in the treatment of gonococcal urethritis

Clinical efficacy in relation to bacterial resistance and its effects on associated *Chlamydia trachomatis* infections

P. M. WATERWORTH,* J. D. ORIEL,† G. L. RIDGWAY,* AND S. SUBRAMANIAN†

From the Departments of *Clinical Microbiology and †Genitourinary Medicine, University College Hospital, London

SUMMARY Seventy-two men with gonococcal urethritis were given a single 300-mg dose of minocycline. The failure rate was 13% and the trial was terminated at an early stage. Failure was correlated with increased resistance of *Neisseria gonorrhoeae* to minocycline.

The activity of penicillin, spectinomycin, erythromycin, tetracycline, sulphamethoxazole, cefuroxime, cefotaxime, rosamicin, thiamphenicol, and piperacillin against *N. gonorrhoeae* were examined *in vitro*. With the exception of spectinomycin, parallel patterns of resistance to the other antibiotics and minocycline were found. Resistance to spectinomycin was not found, confirming the usefulness of this antibiotic in the treatment of gonorrhoea. The incidence of PGU was significantly lower after a single dose of minocycline than in previous studies.

Introduction

Antibacterial drugs have been used for the treatment of gonorrhoea for 40 years, but the emergence of resistant strains of *Neisseria gonorrhoeae* has necessitated the continuous re-evaluation of treatment. Because of resistance, some formerly effective agents—such as the sulphonamides and streptomycin—can no longer be used. Penicillin and its derivatives are still generally effective, but the curative dosage has progressively increased and may before long, even with added probenecid, reach the limit of what is clinically practicable. This problem has been aggravated by the recent advent of the β -lactamase-producing gonococcus, which presents a continuing threat for the future. Sooner or later it will no longer be possible to use penicillin derivatives for the treatment of gonorrhoea, and it is prudent to plan for this eventuality, firstly by *in-vitro* studies of newer antibacterial agents and then by clinical trials.

In view of the importance of *Chlamydia trachomatis* in the aetiology of post-gonococcal urethritis (PGU) (Richmond *et al.*, 1972) and cervicitis (Oriel, 1977), any new antibiotic regimen

would ideally be active against both micro-organisms. The tetracyclines theoretically fulfil this criterion. Earlier studies of the effect of minocycline in gonorrhoea have produced variable results (Duncan *et al.*, 1971; Masterton and Schofield, 1976). We report in this paper a small trial of the effect of a single dose of 300 mg on both gonococcal urethritis in men and the incidence of PGU and the presence of *C. trachomatis*. The high failure rate in the treatment of gonorrhoea led to the curtailment of the clinical trial, but the obvious relationship between treatment failure and raised minimum inhibitory concentration (MIC) for the infecting strain of *N. gonorrhoeae* led us to investigate the current incidence of tetracycline resistance in this area. We also took the opportunity to determine the MIC of a number of newer antibiotics and to compare these with the drugs currently in use in this hospital, namely penicillin and spectinomycin. Erythromycin was included both because of its high activity against *C. trachomatis* and for comparison with rosamicin, which is a new macrolide.

Patients and methods

All the patients attended the Department of Genitourinary Medicine, University College Hospital, London, between October 1977 and January 1978. After local and general examination, a specimen of

Address for reprints: Dr P. M. Waterworth, Department of Clinical Microbiology, University College Hospital, Grafton Way, London WC1

Received for publication 20 March 1979

urethral discharge was taken and spread on a slide for Gram staining and microscopy. A second specimen was inoculated on to a culture medium for *N. gonorrhoeae*. A cottonwool-tipped wire endourethral swab was then inserted 4-5 cm into the urethra and the swab transferred to 2 ml transport medium for *C. trachomatis*. A first-catch urine specimen was examined for evidence of pyuria and serological tests for syphilis were performed.

A presumptive diagnosis of gonorrhoea was made on the basis of the presence of intracellular Gram-negative diplococci on the urethral smear, and patients were then treated with 3 × 100 mg tablets of minocycline given under supervision in the clinic in a single dose. The diagnosis of gonorrhoea was confirmed by culture in every case. Each patient was asked to return for follow-up examinations three, seven, and 14 days after therapy; there was some individual variation in follow-up attendance, but in many cases it was possible to keep patients under surveillance for longer periods. At each attendance symptoms were recorded and microscopy of urethral specimens, culture for *N. gonorrhoeae* and *C. trachomatis*, and examination of the urine for pyuria were repeated.

ASSESSMENT OF PGU

PGU was defined as the presence of ≥20 polymorphonuclear leucocytes (PMN) in at least three fields of a Gram-stained urethral smear examined at a magnification of ×900 (Oriel *et al.*, 1975), or of similar numbers of PMN in urinary sediment of first-catch urine specimens seven or more days after treatment, or of both.

LABORATORY METHODS

Gonococci were isolated from primary cultures on Thayer-Martin medium containing vancomycin, colistin, and trimethoprim. Pure cultures were then stored at -70°C until tested in batches. The first 57 strains were isolated from patients in the clinical trial but after this had been terminated all strains of *N. gonorrhoeae* isolated in the Department of Microbiology were included in the *in-vitro* studies.

MICs were determined by the plate-dilution method using Isosensitest agar (Oxoid) with 5% lysed horse blood added. The inoculum was prepared by growing the organism overnight on Columbia blood agar (Oxoid) and suspending the resulting growth in a small volume of broth until visibly turbid. The plates were inoculated with a multiple replicator using the suspensions diluted approximately 1/100.

All cultures were incubated overnight at 37°C in a CO₂ incubator. A pharmaceutical preparation of penicillin (Glaxo) was used and the remaining drugs were made available as follows: tetracycline,

minocycline, and piperacillin (Lederle Laboratories); sulphamethoxazole (Roche); spectinomycin (Upjohn Ltd); cefuroxime (Glaxo); cefotaxime (Roussel); rosamycin (Schering); erythromycin (Eli Lilly); and thiamphenicol (Infarzan).

Cell culture for *C. trachomatis* was by the method of Reeve *et al.*, (1975) using McCoy cells pretreated with idoxuridine.

Results

EFFECT OF MINOCYCLINE ON GONOCOCCAL URETHRITIS

A group of 72 men with gonococcal urethritis confirmed by culture were treated with a single dose of minocycline 300 mg. Of these men, 59 were heterosexual and 13 homosexual. The drug was well tolerated and no side effects were seen in any of the men. Three patients were reinfected with gonorrhoea during the follow-up period and were removed from the trial, thus leaving a study group of 69 (56 heterosexual and 13 homosexual). Of these, 60 (87%) were cured; 11 were followed up for up to one week (with one negative post-treatment culture result), 27 for up to two weeks (two negative culture results), 14 for up to three weeks (three negative culture results), and eight for up to four weeks (four negative culture results). Nine (13%) of the 69 men had a relapse; all denied further sexual intercourse since treatment. Positive culture results for *N. gonorrhoeae* were obtained from eight of these men on their first follow-up examination seven days after treatment and from one man on the second follow-up examination nine days after treatment, the first post-treatment culture result having been negative.

There was a marked correlation between the clinical results and the MIC estimations for the infecting strains of *N. gonorrhoeae*. Of the eight isolates available for study from men who had a relapse, an MIC of 0.5 µg/ml was found for six, the mean MIC being 0.67 µg/ml; there was no change in MIC for the organisms reisolated from four of these patients. Of the 41 isolates available from men who had been cured, an MIC of 0.5 µg/ml was found for only two, the mean MIC being 0.14 µg/ml.

MIC DETERMINATIONS

The results of the MIC determinations for *N. gonorrhoeae* are presented in the Figure as cumulative percentages of strains inhibited by increasing concentrations. A total of 171 strains were tested against each drug except for piperacillin, cefotaxime, and cefuroxime, for which only 65 strains were tested.

Although minocycline was four times more active than tetracycline against sensitive strains, strains

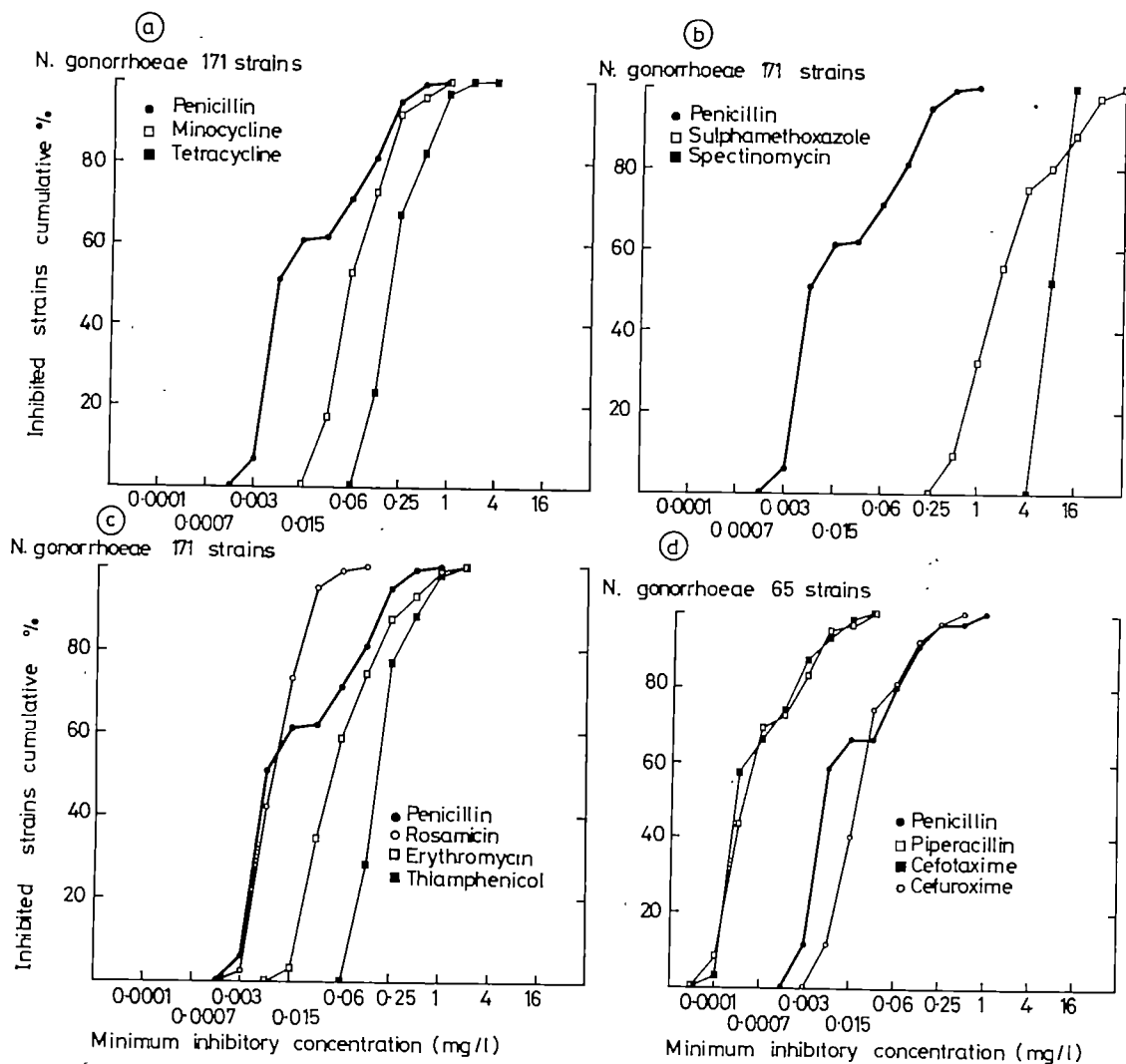


Figure Sensitivity of 171 strains of *N. gonorrhoeae* to (a) penicillin, minocycline, and tetracycline; (b) spectinomycin and sulphamethoxazole; (c) rosamicin, erythromycin, and thiamphenicol; and of 65 strains to (d) piperacillin, cefotaxime, and cefuroxime. Graphs show cumulative percentages of strains inhibited by increasing concentrations of each drug.

resistant to the latter showed a similar or even greater relative increase in the MIC of tetracycline (Figure, a). The curve for penicillin has been included in each graph for comparison and it is obvious that the curves for not only the tetracycline but also for piperacillin, cefotaxime, cefuroxime, sulphamethoxazole, erythromycin, and thiamphenicol all run nearly parallel to this and that for rosamicin is similar but the increase in resistance is less. In contrast, the line for spectinomycin is almost vertical; no resistance was seen and all strains were inhibited by 16 µg/ml.

EFFECT OF MINOCYCLINE ON *C. TRACHOMATIS*

Isolates of *C. trachomatis* were obtained from 10 (17%) of 58 heterosexual men with gonorrhoea but from none of 13 homosexual men. One specimen (from a heterosexual man) was contaminated. None of the three men removed from the trial because of reinfection by *N. gonorrhoeae* yielded chlamydiae before treatment. Of the 10 men who had chlamydia-positive isolates before minocycline therapy, nine had negative results subsequently, the follow-up periods being three days for one man (one negative culture result) eight days for one man (one

negative culture result), nine days for one man (two negative culture results), 14 days for one man (two negative culture results), and 17 days for two men (three negative culture results). *C. trachomatis* was reisolated from one man 14 days after treatment, cell culture having given a negative result after eight days. One other man, chlamydia-negative before treatment, yielded chlamydiae 19 days later; both these men denied sexual intercourse since treatment.

EFFECT OF MINOCYCLINE ON PGU

Of the 72 men with gonorrhoea treated with minocycline, 45 were followed for seven or more days; 36 were heterosexual, of whom six were chlamydia-positive before but not after treatment, one chlamydia-positive both before and after treatment, and one, chlamydia-negative before treatment, yielded chlamydiae subsequently. Nine men were homosexual and were chlamydia-negative throughout. Of the 45 men, eight (18%) developed PGU, including the two who yielded chlamydiae after treatment. None of the five men who were chlamydia-positive before but not after treatment developed PGU.

Discussion

In vitro, minocycline shows considerable activity against *N. gonorrhoeae*; an MIC of ≥ 0.5 $\mu\text{g}/\text{ml}$ was found for only 8% of 171 strains. Although minocycline was four times as active as tetracycline against sensitive strains, those resistant to the latter showed a similar, or greater, relative increase in the MIC of minocycline. The parallel patterns of resistance of *N. gonorrhoeae* to tetracycline, minocycline, erythromycin, rosamicin, and thiamphenicol is interesting and suggests the possibility of difficulties in the use of these antibiotics in the event of treatment failure with penicillin. The lack of such a pattern with spectinomycin confirms the value of this drug in the therapy of penicillin-resistant gonorrhoea.

Minocycline is highly active against the gonococcus but it is clear that treatment with a single dose of 300 mg is unlikely to be successful if the infecting strain shows increased resistance to tetracycline. A failure rate of 13% indicated to us that minocycline in this dosage has only a limited place in the treatment of gonorrhoea. Other workers in Britain have obtained better clinical results, probably because of local variations in the prevalence of tetracycline-resistant strains.

A relationship between treatment failure with single-dose regimens of tetracyclines and the susceptibility of the infecting strains has been described for methacycline and doxycycline by

Wiesner *et al.*, (1973). These, and our own, results show that the clinician who is contemplating a single-dose regimen of a tetracycline for the treatment of gonorrhoea should be aware of the pattern of gonococcal resistance in his own area and keep this under constant review. Whether there has been a general increase in the resistance of *N. gonorrhoeae* to tetracyclines in Britain is not known, but this has undoubtedly occurred in other countries. It seems unlikely that single-dose treatment of gonorrhoea with any tetracycline will prove a satisfactory alternative to present remedies if, and when, these can no longer be used.

The incidence of PGU after minocycline therapy (18%) was markedly lower than we have observed in comparable studies using single-dose ampicillin (37%) or spectinomycin (40%) (Oriel *et al.*, 1976; Oriel *et al.*, 1977). This is probably largely due to the activity of minocycline against *C. trachomatis*, the major cause of PGU. In the present study, chlamydiae were reisolated from only one of eight men after treatment. The periods of follow-up, however, were short, and possibly longer periods of observation would have shown more recurrences; there is evidence from *in-vitro* studies which would support this idea (Ridgway *et al.*, 1978). Nevertheless, from our results chlamydial genital infections might possibly be successfully treated with shorter courses of therapy with minocycline than those presently in use.

Further studies of single-dose treatment of synchronous genital infections with *N. gonorrhoeae* and *C. trachomatis* are desirable, and any new antibacterial agent developed for the treatment of gonorrhoea should be evaluated in this way, with the object of reducing the incidence of PGU and of persistent chlamydial genital infection in women. Rosamicin is highly active against *C. trachomatis*, with an MIC of 0.015 $\mu\text{g}/\text{ml}$, and might be considered for this purpose if the drug is clinically effective in gonorrhoea. Conversely, thiamphenicol, with an MIC for *C. trachomatis* of 0.5 $\mu\text{g}/\text{ml}$, would not be expected to be an effective treatment in this respect.

The high activity of piperacillin (a carbenicillin) and cefotaxime (a cephalosporin) against the gonococcus must be regarded as primarily of academic interest; the place of these antibiotics is more likely to be in the treatment of serious infections with Gram-negative bacilli.

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Vaginal oxytetracycline concentrations

R. N. THIN,* Z. H. AL RAWI,† P. D. SIMMONS,* J. TREHARNE,‡ AND S. TABAQCHALI†

From the Departments of *Genital Medicine and †Medical Microbiology, St Bartholomew's Hospital, and the ‡Virus Laboratory, Department of Clinical Ophthalmology, Institute of Ophthalmology, London

SUMMARY Although tetracycline preparations are widely used in departments of genitourinary medicine, or sexually transmitted diseases clinics, little is known of the concentrations of these preparations in genital secretions. For this reason a microbiological method was used for estimating oxytetracycline concentrations in vaginal secretions. These concentrations varied from 0.6 to 6.5 µg/ml in 19 women who had had sexual contact with a man with non-specific urethritis and who were taking oxytetracycline dihydrate 250 mg four times daily. They were well in excess of the minimum inhibitory concentration of oxytetracycline (0.2 µg/ml) for the strains of *Chlamydia trachomatis* isolated from the patients with positive culture results. Thus, oxytetracycline 250 mg four times daily appears to be a satisfactory regimen for the treatment of chlamydial genital infection in women.

Introduction

The commonest condition diagnosed among men attending departments of genitourinary medicine or sexually transmitted diseases (STD) clinics in England is non-specific or nongonococcal urethritis (NSU) (Department of Health and Social Security, 1977).

There is increasing evidence that in many cases NSU is due to *Chlamydia trachomatis* (Richmond and Oriel, 1978; Schachter, 1978). *In-vitro* studies show that this agent is susceptible to oxytetracycline (Treharne *et al.*, 1977; Ridgway *et al.*, 1978). Even where this organism is not isolated, patients are usually treated with a tetracycline compound (King and Nicol, 1975a; Morton, 1975), so large amounts of tetracycline are prescribed in STD clinics. Furthermore, non-specific genital infection (NSGI) is frequently diagnosed in women either on the basis of chlamydial isolation (Alani *et al.*, 1977) or because of contact with a man with NSU (King and Nicol, 1975b), and the number of such cases is also increasing (Department of Health and Social Security, 1977); many of these women are also treated with tetracycline. As far as we can determine, there is remarkably little information on concentrations of antibiotics in genital secretions, so we considered it worthwhile to estimate oxytetracycline concentrations in the genital

secretions of women who were contacts of men with NSU.

Patients and methods

Twenty women were studied, and at their first attendance general and genital examinations and investigations were carried out as described elsewhere (Burns *et al.*, 1975).

Oral oxytetracycline dihydrate (ICI) 250 mg four times daily for 10 days was prescribed; after the patients had been taking this regimen for seven days they were examined, and vaginal washings were collected using a modification of the method described by Stamey and Condry (1975). A Cusco bivalve speculum was passed, and the vagina was washed out with four 10-ml aliquots of distilled water. The speculum was then removed and rinsed with a further 10-ml aliquot of distilled water, thus giving a total of 50-ml of washings. A 10-ml sample of venous blood was then collected.

Samples were only collected five days after the end of the last menstrual period, and no sample was taken if there was cervical bleeding from any cause. As oxytetracycline produces a plateau, rather than a peak, in the serum concentration two to four hours after ingestion, samples were collected two to four hours after a tablet had been taken.

MICROBIOLOGICAL ASSAY OF VAGINAL OXYTETRACYCLINE CONCENTRATIONS

Preparation of vaginal secretion

The vaginal washout liquid was freeze-dried in an Edwards high-vacuum freeze-drier. The dried

Address for reprints: Dr R. N. Thin, Department of Genital Medicine, St Bartholomew's Hospital, London EC1A 7BE

Received for publication 23 April 1979

specimen was then reconstituted with 5 ml of distilled water, yielding a semi-homogeneous suspension, which was used for the antibiotic assay.

Determination of dilution factor

The dilution factor of the reconstituted secretion was measured using the method described by Stamey and Condry (1975). This is based on measurement of sodium ion concentration $[Na^+]$ in the undiluted and in the reconstituted secretion. The ratio of $[Na^+]$ in the undiluted vaginal secretion to that in the reconstituted secretion represented the dilution factor.

Twenty microlitres of secretion were collected directly from the vagina of 10 patients using a Finpipette and added to 980 μ l of deionised water. The vaginal washout in 50 ml distilled water was then carried out and freeze-dried as described above. Twenty microlitres of the reconstituted suspension of the vaginal washout were treated in the same manner as the pure secretion. One millilitre 10% (w/v in deionised water) trichloroacetic acid (protein precipitating grade BDH) was added to both samples, the mixtures were vortexed and centrifuged at 3000 r/min. for five minutes, and the $[Na^+]$ was estimated in the clear, supernatant liquids using a flame photometer (Evans Electro Selenium Limited).

ASSAY METHOD

Microbiological assay was carried out to estimate concentrations of oxytetracycline in serum and reconstituted vaginal secretion using DST agar (Oxoid) and *Staphylococcus aureus* NCTC 6571 as test organism. Square plates (23 $\times$ 23 cm) were used and 130 ml molten agar (pH 6.8) was poured into each plate; after solidification, the surface was irrigated with 20 ml of 1/100 dilution of an overnight growth of the test organism (approximately 10^7 micro-organisms/ml), the excess liquid was removed, and the plate was dried for 10 minutes at 37°C. Twenty-five holes were punched in the agar and filled with equal quantities of the specimens and standard solutions of oxytetracycline dihydrate in water and serum in concentrations of 0.125, 0.25, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 7.0, 8.0, and 10.0 μ g/ml. The plate was then incubated for 18 hours at 37°C and the diameter of each zone of inhibition measured using a zone reader (Leebrook Scientific and Electronic Eng. Co. Ltd), and the corresponding concentrations were calculated from a standard curve. The concentration of the antibiotic in the reconstituted secretion was multiplied by the dilution factor to determine the concentration in pure secretion.

C. TRACHOMATIS STUDIES

C. trachomatis was cultured on irradiated McCoy cells using the method described by Darougar *et al.*

(1971). The minimum inhibitory concentration (MIC) of oxytetracycline was determined as described by Trehan *et al.* (1977).

Results

The dilution factor of the pure secretion/reconstituted secretion in 10 pairs of samples varied from 3.1 to 6.4 (Table 1) with a standard deviation of 1.02 and coefficient of variation of 20.54; the mean was 4.96, which is similar to the value of 5.0 obtained by Stamey and Condry (1975).

Twenty patients were studied, whose ages ranged from 18 to 39 years with a mean of 25.2 years. All were white; 14 were single and six were married. Each was a sexual contact of a man with NSU and represented all such women who attended seven days after starting therapy during the period of the study and who fulfilled the criteria outlined. The results of the microbiological assay of oxytetracycline in the

Table 1 Determination of the dilution factor of vaginal secretions

Age (years)	$[Na^+]$ reading*		Dilution factor (Pure/reconstituted)
	Pure secretion	Reconstituted secretion	
33	95	19	5.00
43	82	16	5.13
26	87	19	4.58
26	93	30	3.10
21	75	13	5.77
31	90	25	3.60
19	91	19	4.79
21	77	12	6.42
18	84	16	5.25
26	89	15	5.93

*Flame photometer readings

Table 2 Results of microbiological assay of oxytetracycline dihydrate in vaginal secretion and in serum

Case no.	Age (years)	Oxytetracycline concentration (μ g/ml)		
		Pure vaginal secretion	Serum	Ratio*
1	25	0.63	1.70	0.37
2	28	2.00	3.50	0.57
3	26	0.60	2.30	0.26
4	23	1.00	2.70	0.37
5	25	1.25	0.20	6.25
6	23	0.00	2.90	
7	20	1.00	2.25	0.45
8	39	0.60	NS	
9	18	0.60	1.30	0.46
10	29	0.60	2.30	0.26
11	22	0.60	2.00	0.30
12	30	0.60	0.70	0.86
13	21	6.50	3.00	2.17
14	19	4.50	2.20	2.05
15	18	0.60	3.70	0.16
16	32	4.50	3.70	1.22
17	37	3.50	2.50	1.40
18	19	0.63	2.50	0.25
19	23	4.00	3.40	1.18
20	27	2.00	2.70	0.74

*Ratio = vaginal secretion: serum
NS = no blood specimen collected

samples of vaginal secretion and serum are given in Table 2. Oxytetracycline concentrations in vaginal secretion ranged from 0 to 6.5 µg/ml and in serum from 0.2 to 3.7 µg/ml.

C. TRACHOMATIS CULTURES

Specimens for culture of *C. trachomatis* were taken from the cervical os of 17 women; no result was obtained from one specimen because of poor cell growth; six (31.2%) of the remaining cultures gave a positive result. The MICs of oxytetracycline were 0.2 µg/ml for two cultures with positive results from one patient, 0.1-0.2 µg/ml for one from a second patient, and 0.2 µg/ml for one from a third patient. Unfortunately, estimates could not be made on the other two cultures with positive results for technical reasons.

Discussion

C. trachomatis is now regarded as having an important role in the pathogenesis of NSU and NSGI (Richmond and Oriel, 1978). This organism can be cultured from up to half of unselected cases of NSU in men and from 30-35% of women who have had sexual contact with men with NSU (Richmond and Oriel, 1978). The presence or absence of chlamydiae in these women may be significantly associated with its isolation from their contacts (Alani *et al.*, 1977).

The MIC of oxytetracycline for *C. trachomatis* has been reported to vary from 0.03 µg/ml to 0.5 µg/ml for laboratory strains and clinical isolates from patients in London (Treharne *et al.*, 1977; Ridgway *et al.*, 1978). The MIC results (0.1-0.2 µg/ml) obtained in the present study are within this range and with the exception of one patient, the vaginal concentrations of 0.6-6.5 µg/ml exceeded the minimum inhibitory concentrations. All vaginal concentrations exceeded the maximum recorded MIC of 0.5 µg/ml (Treharne *et al.*, 1977).

The exception, Case 6, with a vaginal concentration of zero, had a serum concentration above the mean, and the zero concentration obtained

in vaginal secretion may have been due to a procedural error. Apart from this one case, it is apparent that after treatment with oxytetracycline 250 mg four times daily for seven days adequate concentrations are produced in the vaginal secretion to inhibit *C. trachomatis*. In these cases the vaginal oxytetracycline concentrations were related to the MICs for chlamydiae. Treatment with tetracycline, however, is also important in men with chlamydia-negative NSU and in their female contacts (Thambar *et al.*, 1979).

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Penetrating Sites

Amoxil's ability to penetrate bronchial secretions whether purulent or mucoid, may explain why it has been shown to be more effective than cotrimoxazole or ampicillin in preventing relapse in bronchitis.¹ Amoxil also achieves significantly higher levels than ampicillin in both the maxillary sinus mucosa² and middle ear fluid.³

Powerful Spectrum

Amoxil's broad spectrum of activity extends across the Gram-positive and Gram-negative pathogens responsible for the vast majority of respiratory and urinary infections. In particular, Amoxil is notably active against *H. influenzae*, a predominating pathogen in lower respiratory



infection and otitis media,⁴ and against *E. coli*, the organism largely responsible for simple urinary infection in general practice.

Penicillin Safety

As a penicillin, Amoxil is well tolerated and suitable for most patients, including the elderly and pregnant women. Penicillin hypersensitivity is the only contra-indication.

Prescribing Simplicity

The absorption of Amoxil is virtually unaffected by the presence of food⁵ which adds to the convenience of its tds dosage. And for urinary infections in adults, Amoxil Twin Pack, the complete two dose course of treatment, is simplicity itself.

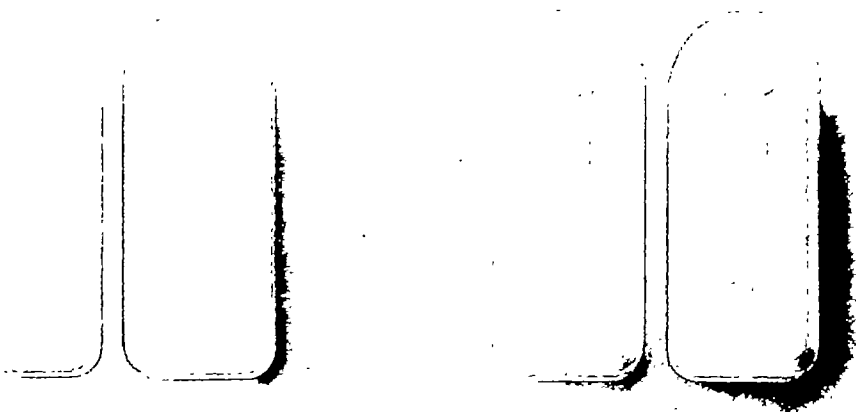
Amoxil

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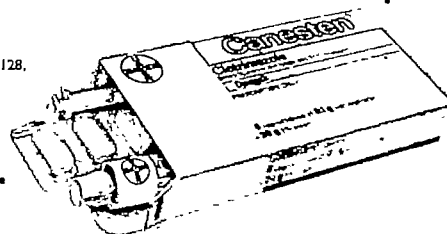
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Metronidazole-resistant *Trichomonas vaginalis*

ARNE FORSGREN AND LARS FORSSMAN

From the Department of Clinical Bacteriology, University of Lund, Malmö General Hospital, Malmö; and the Department of Obstetrics and Gynaecology, University of Gothenburg, East Hospital, Gothenburg, Sweden.

SUMMARY A 36-year-old woman with symptomatic metronidazole-resistant trichomonal vaginitis for 10 years had a total of 22 courses of treatment with either metronidazole or tinidazole according to different schedules. The minimum trichomonicidal concentration of metronidazole for the strain of *Trichomonas vaginalis* isolated from the patient was 160 µg/ml compared with 1.25-10 µg/ml for other freshly isolated strains. The former strain also showed a definitely decreased sensitivity to ornidazole and tinidazole (80 µg/ml). The mechanisms behind the appearance of resistance in this clinical isolate are at present unknown and require further study from the theoretical as well as the therapeutic viewpoint.

Introduction

The treatment of *Trichomonas vaginalis* infections was significantly improved by the discovery of metronidazole in 1959 (Cosar and Julou, 1959). Since that time other antiprotozoal agents among the nitroimidazole derivatives have been used; of these tinidazole and ornidazole are the most powerful (Howes *et al.*, 1970; Sköld *et al.*, 1977). Metronidazole seems to have maintained its efficacy since it was introduced almost 20 years ago, and similar results have been reported for tinidazole (Wallin and Forsgren, 1974). Recently, however, a strain with decreased sensitivity to metronidazole has been isolated (Thurner and Meingassner, 1978). To our knowledge, no previous evidence of the emergence of metronidazole-resistant strains has been reported (Korner and Jensen, 1976; Josey, 1978). This paper describes treatment failure with metronidazole and tinidazole in a patient infected with a metronidazole-resistant strain of *T. vaginalis*.

Case report

A female patient, born in 1943, first visited this clinic because of vaginal trichomoniasis in 1968. Since then she has been seen repeatedly by different doctors in our outpatient department. Trichomonads were found in her vaginal secretions on wet smear

examination at most of her visits. Since 1968 she has had 11 courses of metronidazole at the standard dose of 200 mg three times a day for seven days. On two occasions treatment was given for two or three weeks and on one occasion she had 400 mg three times daily for two weeks. Tinidazole at the standard dose of 2 g as a single dose was given six times; on one occasion a dose of tinidazole 4 g was given. In 1969 nifuratel 200 mg was given three times daily for one week. A cyst of the left Bartholin's gland was excised in 1976, but even after removal of this possible focus of infection her trichomoniasis persisted.

Her husband, who was a ship's mate and went to sea for long periods, also received antitrichomonal treatment at the same time and at the same dosage. The patient denied extramarital sexual contact. In February and in October 1978 her strain (BO) of *T. vaginalis* was analysed for its susceptibility to imidazole derivatives. In June 1978 the patient was given a single dose of tinidazole 2 g and the serum concentration was regularly estimated.

LABORATORY METHODS

Minimum trichomonicidal concentrations were determined (Forsgren, 1972) for the strain (BO) of *T. vaginalis* from the patient and for freshly isolated strains of *T. vaginalis* taken at random from patients' specimens.

The micro-organisms were grown at 37°C in Diamond's medium (Diamond, 1957) containing streptomycin 100 mg, penicillin 1 megaunit, and doxycycline 10 mg/100 ml medium. A suspension of 8×10^4 *T. vaginalis* organisms in 1 ml thioglycollate medium was added to metronidazole, ornidazole, or

Address* for reprints: Dr A. Forsgren, Department of Clinical Bacteriology, University of Lund, Malmö General Hospital, S-214 01 Malmö, Sweden

Received for publication 20 December 1978

tinidazole in serial twofold dilutions in 1 ml saline giving a final concentration of 4×10^4 viable organisms per ml. After incubation for three days at 37°C , 0.2 ml from each test tube was transferred to 4 ml Diamond's medium and the subculture was incubated for five days. Evaluation of the trichomonocidal activity was based on the final result of the subculture and was defined as a concentration ($\mu\text{g/ml}$) in which no viable organisms could be detected by subculture. The determinations were always carried out in duplicate.

The serum concentrations of tinidazole were determined before and at four, eight, 24, and 48 hours after ingestion of tinidazole 2 g on an empty stomach. The sera were heat-inactivated (Forsgren, 1972), diluted in saline, and tested against the sensitive strain of *T. vaginalis* in thioglycollate medium; finally subcultures were performed in Diamond's medium as described above. The serum concentration was calculated by reference to a standard series of tubes containing known concentrations of tinidazole.

Results

The sensitivity to metronidazole of fresh clinical isolates of *T. vaginalis* taken at random from patients' specimens in the routine laboratory is shown in Table 1. The trichomonocidal concentration was 1.25–10 $\mu\text{g/ml}$ with a mean value of 3.75 $\mu\text{g/ml}$, the same concentration as detected with the same technique for clinical isolates in 1972 (Forsgren and Wallin, 1974). The difference in sensitivity for the *T. vaginalis* strain BO was striking (Table 2). The concentration required to obtain a trichomonocidal effect for this strain was 16–128 times higher than for the fresh isolates from patients with therapeutically uncomplicated trichomonal infections.

The serum concentration of tinidazole in the patient after ingestion of tinidazole 2 g showed that absorption of the drug was satisfactory. Serum concentrations of 40 $\mu\text{g/ml}$ and 2.5 $\mu\text{g/ml}$ were detected at four and 48 hours after ingestion respectively.

Table 1 Minimum trichomonocidal concentration ($\mu\text{g/ml}$) of metronidazole for freshly isolated strains of *T. vaginalis*

Strain	Minimum trichomonocidal concentration ($\mu\text{g/ml}$)
1	1.25
2	10
3	5
4	2.5
5	5
6	2.5
7	1.25
8	10
Mean value	3.75

Table 2 Minimum trichomonocidal concentration ($\mu\text{g/ml}$) of three imidazole derivatives for resistant *T. vaginalis* strain BO (I = strain isolated February 1978; II = strain isolated November 1978.)

<i>T. vaginalis</i> strain	Minimum trichomonocidal concentration ($\mu\text{g/ml}$)					
	Metronidazole		Ornidazole		Tinidazole	
	Mean	Range	Mean	Range	Mean	Range
BO I	160	80–320	80	40–160	80	40–160
BO II	160		80		80	

Discussion

Metronidazole, which has been widely used during the last 20 years for treatment of *T. vaginalis* infections, is still generally accepted as being active against all clinical strains of this protozoon (Josey, 1978). Clinical failures in the treatment of trichomoniasis by standard doses of metronidazole have been reported. In most cases, however, these failures were possibly due either to poor absorption of the compound (Kane *et al.*, 1961) or to inactivation of the compound by the vaginal flora (Nicol *et al.*, 1966; McFadzean *et al.*, 1969). In our case, however, no impairment of absorption was evident from study of the serum concentrations after ingestion of a standard dose of tinidazole. The addition of three different antibiotics to the culture medium most probably excluded the possibility of growth *in vitro* of organisms which might have inactivated imidazole derivatives. In addition, no contaminating organisms were seen microscopically.

Some authors have suggested that resistant clinical strains might have appeared (Aure and Gjonnaess, 1959; Arnold, 1966; Turner and Meingassner, 1978), but in only the last of these reports were microbiological confirmatory tests performed. Successful *in-vitro* and *in-vivo* experiments to induce metronidazole resistance in *T. vaginalis* strains, however, have been performed. Carneri *et al.* (1969) and Carneri and Gionnane (1971) observed that after passages of *T. vaginalis* on media containing increasing concentrations of metronidazole the minimum trichomonocidal concentration of metronidazole for the original strain had risen from 0.23 $\mu\text{g/ml}$ to 80 $\mu\text{g/ml}$. On the other hand, in five strains injected into a mouse treated with suboptimal doses of metronidazole and then enriched in culture in the absence of the drug and subjected to 20 of these alternated passages the *in-vivo* resistance was increased by only 4.5 to 14.5 times. Results which agreed with those of Carneri (Carneri *et al.*, 1969; Carneri and Gionnane, 1971) were obtained by Benazet and Guillaume (1971). Resistance to metronidazole could also be induced in *Trichomonas fetus* strains in hamsters infected intravaginally (Actor *et al.*, 1969).

The mechanisms behind the appearance of resistance in the clinical isolate of *T. vaginalis* in this study are not known. It is tempting to speculate, however, that suboptimal doses of metronidazole in vaginal secretions (Paredes and Hawkins, 1973) caused the resistance in the same manner as described above for experimental induction of imidazole resistance. If so, it would seem sensible to give single, high doses of imidazole derivatives (Csonka, 1971; Wallin and Forsgren, 1974; Sköld *et al.*, 1977). It is of interest that eight months after the resistant organisms were first isolated from the patient imidazole-resistant *T. vaginalis* organisms could again be isolated. The woman had in the meantime received one single dose of tinidazole 2 g. Furthermore, the *T. vaginalis* strain showed almost the same high degree of resistance to all the imidazole derivatives tested. The problem of resistance to *T. vaginalis* is worthy of further study, both from the theoretical and the therapeutic viewpoint.

The skilful technical assistance of Mrs Gertrud Hansson is gratefully acknowledged.

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Double-blind comparison of a single dose and a five-day course of metronidazole in the treatment of trichomoniasis*

R. N. THIN, M. A. E. SYMONDS, R. BOOKER, S. COOK, AND F. LANGLET

From St Bartholomew's and Eastern Hospitals, London

SUMMARY Although there have been reports of the results of treating trichomoniasis with metronidazole 2 g in a single dose, no randomised double-blind comparison of this treatment with a multiple-dose regimen has been reported.

In such a comparison, 96 women were treated with metronidazole 2 g in a single dose and 96 women with metronidazole 400 mg twice daily for five days. Forty-eight of 52 women followed for 14 days after the single dose were cured, as were 61 of 66 women followed for 14 days after the start of the five-day regimen. These results compare favourably with previous reports. Side effects were trivial, and we recommend the single 2-g dose, for it is effective, economical, and can be given under supervision in the clinic.

Introduction

The history of the treatment of trichomoniasis was briefly reviewed by Willcox (1977), who noted the gradual reduction in duration of treatment and mentioned that metronidazole 2 g in a single dose gave reasonably satisfactory results. Csonka (1971) appears to have been the first to use this regimen. He compared the findings in 38 patients treated with metronidazole 2 g with those in 49 patients treated with metronidazole 200 mg three times daily for seven days; 82% of the former group and 94% of the latter group were regarded as cured after follow up for three months. Woodcock (1972) and Morton (1972) reported similar cure rates for treatment with metronidazole 2 g in a single dose.

In 1974 cure rates with metronidazole 200 mg three times daily for seven days or 400 mg twice daily for five days were higher in this department at 98.4% in a study of 60 patients treated with each regimen and followed for 14 days after the start of treatment (Thin, unpublished observations). The 2-g single dose was therefore held in reserve for patients with recurrence (that is, reinfection or treatment failure). In 1976 most cases were cured with the single-dose

treatment after there had been recurrence on a longer course. A double-blind comparison of metronidazole in a single 2-g dose with 400 mg of the same drug given twice daily for five days was therefore arranged and the results are reported here.

Patients and methods

PATIENTS

Female patients with vaginal trichomoniasis were studied. All women in whom *Trichomonas vaginalis* was identified were included except those itinerant patients who were unable to attend for two weeks after the start of treatment and pregnant women.

INVESTIGATIONS

A wet preparation was made by mixing vaginal secretion with saline on a slide and examining it under a microscope fitted with a $\times 40$ objective. Another sample was inoculated into trichomonas culture medium (Medical Wire and Equipment Co., Bath).

TREATMENT

Metronidazole (Flagyl, May & Baker Ltd) 400-mg tablets and identical placebo tablets were packed in individual patient packs, numbered, and randomly arranged so that the patient either received metronidazole 2 g immediately, followed by one placebo tablet twice daily for five days, or five placebo tablets immediately followed by metronidazole 400 mg twice daily for five days. No-

*Presented at the Spring Meeting of the MSSVD, May 1979, in Lubbeck, West Germany

Address for reprints: Dr R. N. Thin, Department of Genital Medicine, St Bartholomew's Hospital, London EC1A 7BE

Received for publication 2 April 1979

one in the clinic had access to the treatment code until after treatment and follow up had been completed.

FOLLOW-UP ASSESSMENT

Patients were asked to return seven and 14 days after the start of therapy; wet films and cultures were repeated at each visit.

Although two clinics took part in the study, they are both supervised by the same consultants; the same batch of drugs was used, as were the same laboratory methods and the same batches of culture medium.

In assessing results, treatment failure was defined as reappearance of trichomonads within 14 days of the start of treatment in a patient who denied sexual contact. Reinfection was defined as reappearance of trichomonads in a patient who admitted further sexual contact. Recurrence was treatment failure plus reinfection.

Results

Ninety-six patients were treated with each drug regimen. The patients in the two groups were similar in age distribution, marital state, country of birth, and parity.

Among those treated with metronidazole 2 g, 70 patients were seen at seven days (including one with treatment failure and one who had a reinfection) and 32 patients were seen at 14 days (including two who had a reinfection) (Table). Thus, the total number of failures with this regimen was one (1.0%) of the 32 followed for 14 days; and the total number of recurrences was 19 (17.7% of the 32 followed for 14 days); giving an overall cure rate of 92.3% at 14 days.

Among those treated with metronidazole 400 mg twice daily for five days, 73 patients were seen seven days after the start of treatment, including one with treatment failure but none with reinfection; and 66 patients were seen 14 days after the start of treatment, including two with treatment failure and two with reinfection. The total number of failures was three (4.1% of the 66 followed for 14 days), and the total number of recurrences was five (7.6% of the 66 followed for 14 days); giving an overall cure rate of 92.4% at 14 days.

66 followed for 14 days), giving a cure rate of 92.4%. The marginally better results with the single-dose regimen are not statistically different from those with the five-day regimen.

No patient reported side effects with the five-day regimen. Nausea, vomiting, and dizziness were each reported by one patient with the single-dose regimen. The patient who vomited was withdrawn.

Discussion

The overall cure rate of 48 (92.3%) out of 52 patients followed for 14 days after metronidazole 2 g cannot be compared directly with the findings in other studies as they do not give figures for cures at 14 days. Results from some other studies will be given for approximate comparisons.

In open comparative trials, Csonka (1971) reported cure in approximately 40 (86.9%) of 46 patients given metronidazole 2 g and seen one month later, and Woodcock (1972) reported cure in 51 (85.0%) of 60 patients followed for one to four weeks after treatment with metronidazole 2 g. In a non-comparative study, Morton (1972) reported cure in 97 (82.2%) of 118 women followed for one to three weeks after treatment with the same dose.

Our findings should also be compared with those obtained using other preparations. In a non-comparative study, Jones (1972) reported cure in 149 (81.7%) of 182 women treated with nimorazole 2 g in a single dose and seen at least once after treatment. Nimorazole 2 g in a single dose has given cure rates of 81.46% according to a review published by Sawyer *et al.* (1976). In a comparative trial, Norwick *et al.* (1977) reported cure in 188 (27.7%) of 176 women seen one to three weeks after a single dose of metronidazole 2 g. The cure rate of 61 (92.4%) out of 66 patients followed for 14 days after starting the five-day course compares favourably with cure in 181 (91.4%) of 198 women treated with the same regimen reported by McClean *et al.* (1971). In an open comparative trial, McClean (1972) also reported cure in 182 (82.2%) of 45 patients given the same dose. It should be remembered that we used arbitrary

Table Summary of results

Treatment regimen	No. of patients treated	Duration of follow up (days)		Seven		14		Failure		Reinfection	
		Duration of follow up (days)		Seven		14		Failure		Reinfection	
		No. seen	No. seen	No. seen	%	No. seen	%	No. seen	%	No. seen	%
Metronidazole 2 g immediately	96	70	1	1.4		32		0		2	3.8
400 mg twice daily for 5 days	96	73	1	1.3		66		2	3.0	2	3.0

definitions of treatment failure and reinfection in the present series. Treatment failure in some patients prescribed the five-day course may have been due to failure to take the treatment as directed. Some cases classed as reinfections may have been due to treatment failure.

The results of this double-blind trial compare favourably with these reports, and, in particular, the results of treatment with metronidazole 2 g are better than those quoted by Woodcock (1972) and Morton (1972). Although the only side effects reported were with the single-dose regimen, we believe that they were trivial, and we recommend metronidazole 2 g in a single dose. This has the advantages of economy and the drug can be given under supervision in the clinic.

We are grateful to Dr J. Goulton of May & Baker Ltd, for supplying active and placebo tablets and to Dr J. Farwell of St Bartholomew's Hospital for packing the tablets and arranging the randomisation. We also thank Sister Boutell and the staff of both clinics for their enthusiastic co-operation.

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Epidemiological investigation of patients with vulvovaginal candidosis

Application of a resistogram method for strain differentiation of *Candida albicans*

D. W. WARNOCK, D. C. E. SPELLER, J. D. MILNE, A. L. HILTON,* AND P. I. KERSHAW

From the Bristol Royal Infirmary, Bristol

SUMMARY The resistogram method was applied to 420 isolates of *Candida albicans* obtained from 30 selected patients undergoing treatment for vulvovaginitis. Of these, 16 patients each harboured a particular strain of *C. albicans* which persisted in the mouth or intestinal tract or both. In three of these patients, this strain persisted in the genital tract, and, in eight patients, it later recolonised the genital tract. Fourteen patients harboured more than one strain of *C. albicans*: one failed to respond to treatment and continued to harbour the same strain in the genital tract; in five the original strain later recolonised the genital tract and a second strain remained confined to the mouth or intestinal tract or both; and in three a second strain, present in the intestinal tract, later colonised the genital tract. Each of the male partners of seven patients harboured a strain of *C. albicans* that was identical to the strain, or to one of the strains, that had been isolated from his female partner.

Introduction

The current prevalence of vaginal infection with *Candida albicans* has led to increased interest in the epidemiological aspects of this condition. The source of this infection is an intriguing problem, and its elucidation might be helpful in treating the patient. Most patients harbouring *C. albicans* in the genital tract are also harbouring this fungus in the mouth and anal tract (Hilton and Warnock, 1975), and Miles *et al.* suggested that the digestive tract could be the principal source of vaginal infection (Miles *et al.*, 1977). Furthermore, transmission of *C. albicans* from the male partner might be contributing to the vaginal infection in certain patients (Rodin and Kolator, 1976; Davidson, 1977).

The differentiation of isolates of *C. albicans* has in the past been restricted to the two serological groups of Hasenclever and Mitchell (1961). In practice, most

clinical isolates have been found to belong to one of these groups (Hasenclever and Mitchell, 1963), and this has limited the usefulness of this method for tracing the spread of *C. albicans* infection.

Warnock *et al.* (1979) described a resistogram method for differentiation of strains of *C. albicans* based on differences in the resistance of strains of *C. albicans* to a number of selected chemicals used at critical concentrations. Up to 1092 hypothetical strains of *C. albicans* can be distinguished with this method. The method was tested with isolates from 13 patients undergoing treatment for vaginal infection with *C. albicans*, and it was found that particular strains were consistently present in individual patients, both in different specimens and on different occasions. Furthermore, some patients harboured more than one strain of *C. albicans*.

This paper presents the findings of an epidemiological investigation of 30 patients treated for vulvovaginal infection with *C. albicans*. The strains of *C. albicans* present in the mouth, digestive tract, and genital tract of these patients before and after treatment were characterised by the resistogram method. Isolates of *C. albicans* obtained from the male partners of seven patients were also studied.

*Dr Hilton died recently

Address for reprints: Dr D. W. Warnock, Department of Microbiology, Bristol Royal Infirmary, Bristol BS2 8HW

Received for publication 28 March 1979

Patients and methods

PATIENTS

Thirty non-pregnant patients were selected from among 100 patients taking part in a clinical trial (Milne and Warnock, 1979) and were included in this investigation provided that a minimum of six isolates of *C. albicans* had been obtained and the patient had attended on at least three occasions. The patients were first seen before starting treatment for vulvo-vaginal infection with *C. albicans*; 24 of them had also had such an infection diagnosed on at least one previous occasion (on the basis of both clinical and mycological findings). The patients were aged from 17 to 35 years, and 25 of them were using oral contraception. Before treatment, all the patients had clinical signs of vaginitis and vulvitis (vaginitis was recorded if the vaginal mucosa was reddened or granular; vulvitis was recorded if the vulva was reddened, swollen, fissured, or ulcerated) together with a positive result to a vaginal culture for *C. albicans*. No other infection was diagnosed. No patient had taken antibiotics or received local antifungal treatment during the preceding fortnight.

MEDICATION

The patients were treated with one clotrimazole vaginal pessary (100 mg) per night for six consecutive nights. Seventeen patients were given additional oral nystatin treatment: four tablets (500 000 IU per tablet) per day for 10 consecutive days. The male partners were not treated. Patients who required further treatment were given one clotrimazole vaginal pessary per night for 12 nights plus four nystatin tablets by mouth per day for 10 days.

FOLLOW-UP ASSESSMENT

The patients returned for assessment on at least two occasions after the initial course of treatment had been completed. Patients who returned with clinical signs of vaginitis or vulvitis and had a positive result to a vaginal culture were given a further course of treatment. Vaginal, oral, and anal swabs were taken at each visit, placed in transport medium (Oxoid Trichomonas Medium No. 2), and sent for mycological investigation. The patients also provided a faecal specimen on each occasion.

The male partners of seven patients attended for investigation. Oral, urethral, and sub-preputial swabs were taken from these men, placed in transport medium, and sent for investigation.

ISOLATION AND IDENTIFICATION

Vaginal, oral, anal, and faecal specimens were inoculated on to plates of glucose peptone agar containing 0.05 mg chloramphenicol per ml and

incubated at 37°C for 48 hours. Identification of isolates was based on morphological and biochemical tests according to English (1974).

RESISTOGRAM DETERMINATION

This was performed according to the methods of Warnock *et al.* (1979).

The resistogram of each *C. albicans* strain is a list of the chemicals to which that strain is resistant. Each chemical is allotted a letter of the alphabet so that if a strain is resistant to chemical B, the letter B is included in the description of that strain. If a strain is resistant to all the chemicals used, its resistogram is given as A B C D E F. If chemicals A and D inhibit a strain, the resistogram of that strain is given as B C E F. If a chemical causes partial inhibition of a strain, this is denoted by the letter bracketed.

If two isolates demonstrate a major difference in resistance to one or more chemicals—for example, A B C D E and B C D E—or minor differences in resistance to two or more chemicals—for example, A B C D E and (A)(B) C D E or (A)(B) C D E and C D E—the isolates are considered to belong to distinct strains.

Results

The findings for the 30 female patients in this investigation are summarised in Table 1: 420 colonies of *C. albicans* from the cultures of 420 specimens were subcultured for characterisation. In 26 patients the initial course of treatment eliminated the genital tract infection; in 10 of these patients, genital tract recolonisation did not occur. In the other 20 patients, the genital tract remained colonised or became recolonised once treatment had ceased. Thirteen of these 20 patients developed further clinical signs of genital infection and required a second course of treatment. Five of these 13 patients later received a third course of treatment, and one patient required a fourth course of treatment. In all, 16 patients (Patients 1-5 and 11-21) harboured one particular strain of *C. albicans* throughout the period of investigation, and the other 14 patients (Patients 6-10 and 22-30) harboured two distinct strains.

Five of the 10 patients (Patients 1-5) in whom genital colonisation did not recur harboured a particular strain of *C. albicans* in both the digestive tract and the genital tract. In three of the five other patients in whom genital colonisation did not recur (Patients 6, 7, and 8), two strains of *C. albicans* were isolated before treatment. The strain that was isolated from the anus and genital tract of these three patients before treatment did not reappear after treatment. In Patients 9 and 10, a second strain appeared in the digestive tract after treatment.

Table 1 Resistograms of 480 isolates of *Candida albicans*

Patient no.	Resistogram of strain isolated	Site(s) of isolation at different visits						
		Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
Each harbouring a single strain of <i>C. albicans</i> (total of 49 isolates)								
1*	C D E (F)	VA FM		AFM	(4)	AFM (6)		
2*	C (D) E (F)	VA FM	FM (3)	FM (5)	F (8)	FM (10)		
3*	(A) C D E (F)	VA FM	AFM (3)			AFM (16)		
4*	(A) C D E (F)	VA FM	FM (2)	F (4)				
5	(A) B C D E (F)	VA FM	AFM (2)	FM (4)	FM (8)			
Each harbouring two strains of <i>C. albicans</i> (total of 42 isolates)								
6	(A) C (D) E (F)	VA FM	FM (3)	FM (5)				
7	C (F)	VA M	F (2)	M (4)	FM (8)	M (10)		
8	C D (E) (F)	VA F		F (4)	A M (8)			
9*	(B) C (D) E (F)	VA M	AFM (3)	AF (5)	F (8)			
10*	(B) C (D) E (F)	VA FM	FM (2)	A M (4)	M (8)			
	(A) C D E (F)			F (4)	F (8)			
Each harbouring a single strain of <i>C. albicans</i> (total of 120 isolates)								
11	(B) C D E (F)	VA F	VA (2)	V (6)	VA M (8)	V (10)		
12*	(C) C D E (F)	VA V	VA (2)	VA (4)†	V (8)			
13*	(A) C D E (F)	VA M	VA (2)	VA (4)†	VA (10)	VA M (18)		
14*	(A) C (D) E (F)	VA FM	FM (3)	V FM (5)				
15	(A) B C (D) E (F)	VA FM	FM (2)	V F (6)	FM (8)			
16	(B) C (D) E (F)	VA FM	FM (2)	VA FM (4)†				
17*	(C) D E (F)	VA F	F (3)	VA F (5)†				
18	(A) B C D E (F)	VA FM	AFM (2)	AFM (8)		FM (10)		M (18)
19	(A) C (D) E (F)	VA FM	FM (2)	AFM (5)	FM (9)			A M (18)
20	(B) C D (E) (F)	VA F	F (3)	AFM (9)	AF (9)	VA M (14)†		VA FM (20)†
21*	(C) D E (F)	V FM	M (2)	F (4)		M (10)		V FM (20)
Each harbouring two strains of <i>C. albicans</i> (total of 52 isolates)								
22*	(B) C D E (F)	VA M	VA (2)	V (4)				
23*	(B) C (D) E (F)	V	FM (2)	FM (4)	FM (8)			
24	A C D E (F)	VA FM	A (2)	AFM (4)	VA M (9)			
25*	(B) C (D) E (F)	V FM	F (2)	FM (4)	VA F (8)†	VA (12)		
	(C) E	VA F	F (2)	FM (4)	V (9)	FM (12)		
	(A) B C (D) E (F)			FM (4)	VA F (15)†	VA F (15)	M (18)	VA (24)†
								F (24)
Each harbouring two strains of <i>C. albicans</i> (total of 57 isolates)								
26	C D E	VA		VA (6)†		VA F (12)†		
27	(A) B C (D) E (F)	VA				VA (18)		VA (21)†
28*	(A) C D (E)	VA FM	F (3)			VA (19)†		
29*	(A) C D E (F)	VA FM	AFM (2)	M (4)	AF (9)†			
	(A) C D E (F)	VA F	A (2)	VA (4)	V (9)†			
30	A C D E (F)	VA	F (2)	VA M (4)	M (8)	VA F (14)		A M (25)
	(B) C (D) E (F)					F (18)		FM (20)
						VA (18)		VA (20)†

Figures in parentheses indicate time of visit (weeks)

* Patient given oral treatment

† Patient given further treatment

V = vaginal isolate; A = anal isolate; F = faecal isolate; M = oral isolate

Eleven of the 20 patients (Patients 11-21) in whom the genital tract remained colonised, or became recolonised, harboured a particular strain of *C. albicans* throughout the period of investigation. Five of these patients had no further clinical signs of genital infection and did not need further treatment.

Four patients (Patients 22-25) in whom the genital tract remained colonised or became recolonised, harboured two strains of *C. albicans* before treatment. In these patients it was the strain that had been isolated from the genital tract before treatment that later returned. Two of these patients developed signs of genital infection and required a further course of treatment.

Five patients (Patients 26-30) in whom the genital tract became recolonised harboured one strain of *C. albicans* before treatment but later acquired a second strain. In Patient 27, the original strain reappeared in the anus and genital tract and the second strain remained confined to the digestive tract. In Patients 28, 29, and 30, the original strain remained in the digestive tract but a second strain appeared and colonised the anus and genital tract.

Patient 26 received four courses of treatment and on each occasion it was the same strain that was isolated from both the genital tract and the digestive tract. A second strain did appear in the mouth of this patient; this strain had earlier been isolated from the mouth of the male partner of this particular patient (Table 2).

The male partners of seven female patients were studied; 12 isolates of *C. albicans* were obtained for characterisation (Table 2). Each male patient was harbouring a strain of *C. albicans* that was identical to the strain, or one of the strains, that had been isolated from his female partner.

Table 2 Comparison of resistograms of isolates of *Candida albicans* from seven pairs of patients

Female patient no.	Resistogram of strain isolated	Site(s) of isolation			
		Female patient		Male patient	
2	C (D) E (F)	FM	(3)	USM	(3)
4	(A) C D E (F)	VA FM	(0)	S	(0)
11	(B) C D E (F)	VA F	(0)	M	(0)
13	(A) C D E (F)	VA M	(0)	M	(0)
16	(B) C (D) E (F)	VA FM	(0)	USM	(0)
26	(A)(B) C (D) E (F)	M	(21)	M	(12)
30	(B) C (D) E (F)	VA	(20)	US	(20)

Figures in parentheses indicate time of visit (weeks)
 U = urethral isolate; S = sub-preputial isolate; M = oral isolate
 VAF = as Table 1

Discussion

The findings of our previous investigation (Warnock *et al.*, 1979) suggested that particular strains of *C. albicans* were present in individual patients. One of

the patients studied harboured more than one strain of *C. albicans*, and this raised the problem of the number of colonies from each clinical specimen that needed to be tested to detect all the strains that might be present. In its present form, the resistogram method permits 15 isolates to be tested on each occasion. In this investigation, the need to test isolates from all the specimens obtained from a particular patient on the same occasion restricted the number of colonies that could be sampled from each clinical specimen. It cannot, therefore, be assumed that if a strain was not detected in a particular specimen on a particular occasion that strain was not present in that specimen. It should be emphasised that other methods of strain differentiation share this fundamental problem.

There have been no reports that *C. albicans* can become resistant to the antifungal preparations used to treat vaginal candidosis. It has been assumed, therefore, that the failure of some patients to respond to treatment, and the need of others for prolonged or repeated courses of treatment, is due (in part at least) to reinfection from their partner or from their own intestinal tract. Furthermore, the recurrence of genital infection in some patients may possibly be a consequence of incomplete eradication of the initial vaginal infection.

The presence of *C. albicans* in the genital tract is often associated with its presence in the digestive tract (Hilton and Warnock, 1975; Miles *et al.*, 1977). Our findings confirm this association and suggest that most, if not all, patients with genital infection are also harbouring the same strain of *C. albicans* in the digestive tract. The fact that most of our patients harboured the same strain of *C. albicans* in the mouth and intestinal tract suggests that the mouth might be contributing to continuing intestinal colonisation.

The findings suggest that, in patients with profuse vaginal discharge, contamination from the genital tract could be contributing to anal colonisation. Of the seven patients (Patients 6-8 and 22-25) harbouring two distinct strains of *C. albicans* before treatment, five were harbouring one strain in both the anus and the genital tract, and the second strain was present in the mouth or intestinal tract or both. In three (Patients 6, 7, and 8) of these five patients, the strain present in the anus and genital tract before treatment did not reappear after treatment. In the other patients (Patients 22 and 25), the strain that was isolated from the anus and genital tract before treatment later returned; this strain was not isolated from the mouth or intestinal tract. In another patient (Patient 24), the strain that was isolated from the genital tract before treatment was later isolated from the anus and genital tract.

The intestinal tract appears to be a possible source for vaginal reinfection with *C. albicans*. Nine (Patients 14-21 and 26) of the 16 patients in whom genital tract colonisation recurred had continued to harbour the original strain of *C. albicans* in the digestive tract after the initial course of treatment. This strain later recolonised the genital tract. In another patient (Patient 29), it was a second strain, present in the digestive tract, that later appeared in the genital tract.

Incomplete eradication of the initial vaginal infection appears to have occurred in a number of our patients. Patients 11, 12, 13, and 22 were still harbouring the same strain of *C. albicans* in the genital tract at their first visit after completion of treatment. In Patients 23, 24, 25, and 27, the original strain isolated from the genital tract was later isolated from the anus and genital tract but not from the intestinal tract (in the case of Patient 23 the strain concerned was later isolated from the mouth). If the anal colonisation in these patients was derived from the genital tract then the initial vaginal infection might not have been eliminated.

It has sometimes been suggested (Rodin and Kolator, 1976; Davidson, 1977) that *C. albicans* is transmitted to or from the male partner. Our findings support this suggestion; identical strains of *C. albicans* were isolated from the genitalia of three pairs of patients, and in the other four pairs of patients studied both partners harboured the same strain in the mouth or digestive tract. Most of our female patients admitted orogenital contact, which suggests that transmission of *C. albicans* from the

mouth might be contributing to the genital colonisation in some patients.

This investigation has shown that most patients with vulvovaginal candidosis harbour the same strain of *C. albicans* in the mouth, intestinal tract, anus, and genital tract and that the male partner often harbours the same strain. Our findings suggest that the failure of patients to respond to treatment is often due to incomplete eradication of the initial infection. In other patients, recurrent vaginal infection is due, in part at least, to reinfection from the intestinal tract or from the male partner.

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Effect of simultaneous oral and vaginal treatment on the rate of cure and relapse in vaginal candidosis

J. D. MILNE AND D. W. WARNOCK

From the Bristol Royal Infirmary, Bristol

SUMMARY One hundred patients with vulvovaginal candidosis were entered in a double-blind trial to compare the effect of six days' local treatment with clotrimazole with that of the same treatment plus 10 days' oral treatment with nystatin. No significant differences were detected in the rate of cure or relapse between the treatment groups. The cure rate was lowest and the relapse rate highest in whom vaginal candidosis had last been diagnosed during the preceding 12 months.

Introduction

Vaginal infection with *Candida albicans* is a common condition (Oriel *et al.*, 1972; Hilton and Warnock, 1975). Most, if not all, patients with this infection also harbour this fungus in their digestive tract (Hilton and Warnock, 1975; Miles *et al.*, 1977), and it has been suggested that the failure of a significant proportion of such patients to respond to local treatment, and the need of others for prolonged or repeated courses of treatment, is due—in part at least—to reinfection from their intestinal tract. Indeed, Miles *et al.* (1977) suggested that the intestinal reservoir must be eliminated if the genital infection is to be cured.

Oral treatment with nystatin has been shown to reduce intestinal colonisation with *C. albicans* (Seelig, 1968). For this reason, such treatment is often recommended as an adjunct to vaginal treatment in patients with recurrent genital infection (Nicol, 1971; Cartwright, 1978). In fact, it has not been proved that simultaneous treatment of the intestinal tract prolongs freedom from reinfection, and it has not been established that the intestinal tract is recolonised once oral treatment is discontinued. In this investigation we have evaluated the effect of simultaneous oral treatment with nystatin on the rate of cure and relapse in patients presenting with vulvovaginal candidosis.

Address for reprints: Dr D. W. Warnock, Department of Microbiology, Bristol Royal Infirmary, Marlborough Street, Bristol BS2 8HW

Received for publication 28 March 1979

Patients and methods

STUDY POPULATION

The patients studied were 100 non-pregnant women attending the Special Clinic of this hospital. Vaginal candidosis had been diagnosed in 67 of them on at least one previous occasion (on the basis of both clinical and mycological findings), and, at the time of admission to this investigation, all had clinical signs of vulvovaginitis together with a positive result to a vaginal culture (see below). None of the patients had taken antibiotics or received local antifungal treatment during the preceding two weeks.

TREATMENT GROUPS

Patients were allocated in random order to one of two groups for treatment: those in group 1 were treated with one clotrimazole vaginal pessary (100 mg) daily for six consecutive days plus one oral nystatin tablet four times daily for 10 consecutive days; and those in group 2 were treated with one clotrimazole vaginal pessary daily for six days plus one identical oral placebo tablet four times daily for 10 days. The composition of the two groups of patients was similar (Table 1).

FOLLOW-UP ASSESSMENT

All patients were requested to return for assessment at two, four, and eight weeks after treatment was begun. On each occasion symptoms and clinical signs of vaginitis (the vaginal mucosa was reddened or granular), vulvitis (the vulva was reddened, swollen, fissured, or ulcerated), and discharge were noted. High vaginal swabs were taken at each visit, placed in transport medium (Oxoid Trichomonas Medium No. 2), and sent for mycological investigation. The

Table 1 Characteristics of the 100 patients studied

Characteristics	Treatment group	
	Group 1*	Group 2†
No. of patients	50	50
Age range (years)	17-35	17-35
Mean age	22.6	23.9
No. using oral contraception	39	36
Time since last diagnosed vaginal infection:		
1-12 months	17	17
13-36 months	17	16
>36 months	16	17
Vaginal isolate before treatment:		
<i>C. albicans</i>	48	48
<i>C. tropicalis</i>	2	2
Faecal isolate before treatment:		
<i>C. albicans</i>	40	38
<i>C. tropicalis</i>	1	0

* Group treated with clotrimazole vaginal pessary plus oral nystatin

† Group treated with pessary alone

patients also provided a faecal specimen on each occasion. Clinical observations were all made by one observer.

Vaginal and faecal specimens were inoculated on to plates of glucose peptone agar containing 0.05 mg chloramphenicol per ml and incubated at 37°C for 48 hours. Identification of isolates was based on morphological and biochemical tests according to English (1974).

STATISTICAL METHOD

The results were analysed with the χ^2 test (with Yates's correction).

Results

The patients were assessed two weeks after treatment was begun and the findings are summarised in Table 2. In this Table, the patients have been classified as follows: clinical and mycological cure means no clinical signs of infection and a negative vaginal culture result; mycological failure means no clinical signs of infection and a positive vaginal culture

result; clinical and mycological failure means clinical signs of infection and a positive vaginal culture result.

The patients in each treatment group were subdivided into three subgroups: (a) those in whom vaginal candidosis had been diagnosed during the preceding 12 months; (b) those in whom the condition had last been diagnosed during the preceding 13 to 36 months; (c) and those in whom the condition had not been diagnosed during the preceding 36 months.

The cure rate was lowest, and the failure rate highest, in the group of patients in whom vaginal candidosis had been diagnosed during the preceding 12 months. No significant differences could be detected in the cure rate or failure rate between the treatment groups.

VAGINAL CULTURES

The results of vaginal cultures performed at two, four, and eight weeks are presented in Table 3 as the number of patients with positive culture results seen on each occasion. Not all the patients returned at four or eight weeks; 12 patients with negative vaginal culture results at two weeks failed to attend at four or eight weeks and 20 patients with negative vaginal culture results at four weeks failed to return at eight weeks.

At two weeks, eight patients in group 1 (those treated with clotrimazole pessary plus oral nystatin) and 11 patients in group 2 (those treated with pessary alone) still had positive vaginal culture results (Table 3). Thirteen of these patients required further treatment. Up to eight weeks a further nine patients in group 1 had become reinfected; four of these patients had returned with symptoms and clinical signs of infection and had needed further treatment. Seven patients in group 2 who had returned at four or eight weeks had become reinfected; three of these patients required further treatment. No significant difference in the reinfection rate was detected between the two treatment groups at four or eight weeks.

FAECAL CULTURES

The number of patients in each treatment group with positive faecal culture results before treatment and at two, four and eight weeks is presented in Table 4. In all, 79 patients had positive faecal culture results before treatment; 20 patients in group 1 and 24 patients in group 2 had positive faecal culture results before treatment and again two weeks later. No significant differences were detected between the two treatment groups before treatment or at two, four, or eight weeks.

Table 2 Effect of treatment on 100 patients assessed at two weeks

Treatment group	Time since last diagnosed infection	No. of patients		
		Clinical and mycological cure	Mycological failure	Clinical and mycological failure
Group 1*	1-12 months	12	3	2
	13-36 months	15	1	1
	>36 months	15	1	0
Group 2†	1-12 months	11	3	3
	13-36 months	14	1	1
	>36 months	14	2	1

* Group treated with clotrimazole vaginal pessary plus oral nystatin

† Group treated with pessary alone

Table 3 Number of patients with positive vaginal culture results at two, four, and eight weeks

Treatment group	Time since last diagnosed infection	No. of patients with positive results at (weeks)		
		2	4	8
Group 1†	1-12 months	5 (4)*	2 (1)	0
	13-36 months	2 (2)	3 (1)	1 (1)
	>36 months	1 (0)	3 (1)	0
Group 2‡	1-12 months	6 (3)	1 (1)	0
	13-36 months	2 (1)	1 (0)	2 (2)
	>36 months	3 (3)	1 (0)	2 (0)

* Numbers in parentheses indicate no. of patients with clinical signs of infection requiring further treatment

† Group treated with clotrimazole vaginal pessary plus oral nystatin

‡ Group treated with pessary alone

Table 4 Number of patients with positive faecal culture results before treatment and at two, four, and eight weeks

Treatment group	Before treatment		Cumulative no. of patients with positive results at (weeks)		
	Faecal culture result	No. of patients	2	4	8
Group 1*	Positive	41	20	25	27
	Negative	9	0	3	3
Group 2†	Positive	38	24	29	29
	Negative	12	2	3	3

* Group treated with clotrimazole vaginal pessary plus oral nystatin

† Group treated with pessary alone

Table 5 Faecal findings in 35 patients with positive vaginal culture results at two, four, or eight weeks

Treatment group	Faecal	Culture results								
		Before treatment			At 2 weeks			At 4 weeks		
		Faecal		Vaginal	Faecal		Vaginal	Faecal		Vaginal
		+	-		+	-		+	-	
Group 1*	Positive	2	4	8	6	0	8	1	0	1
	Negative	0	2		0	2		0	0	
Group 2†	Positive	5	3	11	2	1	3	2	1	4
	Negative	1	2		0	0		0	1	
Total		8	11	19	8	3	11	3	2	5

+ Positive - negative

* Group treated with clotrimazole vaginal pessary plus oral nystatin

† Group treated with pessary alone

The mycological findings in the 35 patients with positive vaginal culture results at two, four, or eight weeks are summarised in Table 5. Eleven of the 19 patients with positive vaginal culture results at two weeks had negative faecal culture results. Seven of the nine patients in group 1 with positive vaginal culture results at four or eight weeks also had positive faecal culture results. Two patients in this group had negative faecal culture results on both occasions. Four of the seven patients in group 2 with positive vaginal culture results at four or eight weeks also had positive faecal culture results.

Discussion

Many clinicians consider simultaneous oral treatment with nystatin to be a useful adjunct to the local

treatment of patients with persistent vulvovaginal candidosis on the assumption that such treatment will help to prevent reinfection from the intestinal tract. Most of the patients admitted to this investigation had positive faecal culture results as did most of the patients who became reinfected. In this respect, our findings confirm those of another recent investigation (Miles *et al.*, 1977).

Our findings indicate that simultaneous oral and vaginal treatment did not bring about a higher cure rate. This is in accordance with the findings of Velupillai and Thin (1977), who detected no significant difference between the effect of combined local and oral nystatin treatment compared with local nystatin treatment alone. The oral treatment regimen adopted in this investigation has been recommended for the treatment of patients with long-standing

vaginal candidosis (Nicol, 1971; Cartwright, 1978), but it failed to eradicate faecal colonisation in a substantial proportion of our patients. It is interesting that the number of patients with positive faecal culture results in the group treated with pessary alone decreased during treatment. The reason for this apparent loss of intestinal colonisation is uncertain but the antifungal preparation may have contaminated the anal tract and so reduced or eliminated faecal colonisation.

It cannot be said for certain that the patients who responded to treatment but later relapsed derived their later infection from their intestinal tract. Eight of the 11 patients with positive vaginal culture results at four weeks and three of the five patients with positive vaginal culture results at eight weeks did have positive faecal culture results. This does suggest that the intestinal tract may be a source of vaginal infection, but other sources of infection, including the male partner (Rodin and Kolator, 1976), cannot be discounted.

In conclusion, simultaneous oral treatment with nystatin did not increase the cure rate nor did it decrease the relapse rate in our patients. Prolonged oral treatment with nystatin has helped a number of patients with chronic vulvovaginal candidosis, but this investigation has shown that intestinal colonisation often recurs once oral treatment has been discontinued. If the mouth is the principal source of intestinal colonisation, then eradication of this reservoir of infection might help to reduce the spread of *C. albicans*. Intermittent prophylactic treatment could be the best means of preventing

vulvovaginal infection in predisposed patients. The beneficial effect of intermittent vaginal treatment has been reported (Davidson and Mould, 1978), but the role of intermittent intestinal treatment remains to be evaluated.

The authors are indebted to the late Dr A. L. Hilton and Dr C. S. Good for their help in the design of this investigation, to the staff of the Special Clinic for their co-operation, and to Bayer UK Limited for financial support.

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Sexual transmission of hepatitis B surface antigen Infection of husbands by HBsAg carrier-state wives

N. INABA, R. OHKAWA, A. MATSUURA, J. KUDOH, AND H. TAKAMIZAWA

From the Department of Gynaecology and Obstetrics, Chiba University School of Medicine, Chiba, Japan

SUMMARY The husbands of 68 hepatitis B surface antigen (HBsAg) carrier-state wives were tested for the presence of hepatitis B surface antibody (HBsAb) and antigen (HBsAg) to assess possible transmission of HBsAg. Eight (11.8%) of the 68 husbands gave negative results for HBsAg and 22 (32.4%) positive results for HBsAb. Furthermore, eight (26.7%) of 30 husbands with presumed HBsAg transmission from their wives developed acute viral hepatitis after marriage, and e antigen (eAg) was detected in the serum from all eight wives. Although HBsAg was not detected in specimens of sputum and cervical mucus of carrier-state women by reverse passive haemagglutination, it was detected in the vaginal discharge of women during days 1-6 of the menstrual cycle at a rate ranging from 20-60%. Thus, sexual transmission of HBsAg seems to occur, particularly if sexual contact takes place during or immediately after menstruation.

Introduction

Studies on the prevalence of hepatitis B surface antibody (HBsAb) in populations with different levels of promiscuity have suggested the possibility of sexual transmission of hepatitis B surface antigen (HBsAg) (Hersh *et al.*, 1971; Heathcote and Sherlock, 1973; Papaevangelou *et al.*, 1974; Froesner *et al.*, 1975). HBsAb studies on Athenian prostitutes by Papaevangelou (1974) support this hypothesis, and Froesner *et al.* (1975) reported that the prevalence of HBsAb in West German prostitutes tended to increase with age and length of prostitution. Another study performed in Cali by Adam *et al.* (1974), however, showed no significant HBsAb percentage difference between the sera of prostitutes and of control populations.

We studied the incidence of HBsAg and HBsAb in husbands of HBsAg-carrier-state wives to assess possible sexual transmission of HBsAg.

Patients and methods

Thirteen thousand pregnant women attending 35 obstetric institutions in Chiba Prefecture were screened for HBsAg and HBsAb. Of 195 HBsAg-positive pregnant women and their husbands 68 were

selected (as indicated below) for study and followed up for periods ranging from one to two years. The women were designated as asymptomatic HBsAg carriers on the following criteria: (1) the presence of serum HBsAg for at least six months; and (2) the absence of any clinical manifestations of liver disease or laboratory abnormalities in liver function.

The husbands were interviewed regarding premarital and extramarital sexual partners, and living parents and siblings were tested for serum HBsAg or HBsAb either at our hospital or, in a few cases, at other institutions. Most husbands participating in this study had had no premarital sexual partners other than their wives and no extramarital intercourse. All parents and siblings tested gave negative results for HBsAg and HBsAb. Husbands who had episodes of acute viral hepatitis shortly after marriage had no family history of acute hepatitis, hepatoma, or liver cirrhosis.

Sputum samples were obtained repeatedly from the 68 HBsAg-positive women before and after delivery. Specimens of cervical mucus were collected from 18 postpartum women before menstruation restarted. Samples of vaginal discharge from five postpartum women were obtained every day for 30 days and tested for HBsAg. Samples of sputum, cervical mucus, and vaginal discharge were diluted (1/1) with Ringer's solution, centrifuged at $3000 \times g$, and stored at -20°C . Blood samples were obtained from the 68 husbands. For comparison 28 HBsAg-negative pregnant women and their husbands were also tested.

Address for reprints: Dr N. Inaba, Behring Institute, 3520 Marburg/Lahn, West Germany

Received for publication 7 March 1979

Serum samples were screened for HBsAg at dilutions of 1/2, 1/4, 1/8, 1/16, and 1/256. Titres over 1/4 were considered to be positive. Testing for HBsAg was carried out by immune adherence haemagglutination (IAHA) (Mayumi *et al.*, 1971) and reverse passive haemagglutination (R-PHA) (Schuurs and Kaoki, 1974). HBsAb was determined by passive haemagglutination (PHA) (Vyas and Shulman, 1974). Positive results were confirmed by absorption with HBsAg or human HBsAb. Antigen and antibody subtyping was performed by the haemagglutination inhibition (HI) technique (Imai *et al.*, 1974). Carrier-e-system was determined by the micro-Ouchterlony method (Magnius and Espmark, 1972).

Results

Of 68 husbands with HBsAg carrier-state wives, 30 (44.2%) were either HBsAg-positive (11.8%) or HBsAb-positive (32.4%). Antigen-subtypes or antibody-subtypes were identical in all 30 pairs. No significant difference in adr:adw ratio was found among the groups with and without HBsAg transmission (Table 1).

Of the husbands of the 28 HBsAg-negative pregnant women, none was HBsAg-positive and only one was HBsAb-positive.

Serum eAg was found in samples from four of eight HBsAg-positive women whose husbands were

HBsAg-positive and in those from 17 (77.3%) of 22 wives with HBsAb-positive husbands. Three (8%) of 38 women whose husbands were HBsAg-negative or HBsAb-negative had serum eAg, and 23 (60.5%) women had serum eAb. Eight (26.7%) of 30 husbands with presumed HBsAg transmission from their wives had a history of acute viral hepatitis shortly after marriage and eAg was present in the sera of all eight wives (Table 1). No other source of infection was detected.

HBsAg was detected by R-PHA neither in the sputum from the 68 HBsAg carrier-state women nor in the cervical mucus of the 18 women examined before the start of menstruation. HBsAg was detected in 20-60% of samples collected during the first six days of the menstrual cycle (Table 2) but not in any sample collected later in the cycle.

Discussion

Although Piccinino (1975) found the incidence of HBsAg to be higher among children than among their parents, according to Inaba *et al.* (1977) the vertical transmission rate of HBsAg in Japan is approximately 38%. Inaba *et al.* (1977) also showed that of 13000 pregnant women screened for HBsAg and HBsAb, 195 (1.5%) were HBsAg-positive, and 1560 (12%) HBsAb-positive. Among male blood donors in Tokyo, the incidence of HBsAg was approximately 2.3% (Okochi *et al.*, 1970) and that of

Table 1 Incidence of HBsAg transmission from HBsAg-carrier-state wives to their husbands and correlation with e-system and subtype in women's sera

Husbands' serum results		Results in serum of HBsAg-carrier wives										
		e-system						Subtype				
		eAg +		eAb +		eAg - and eAb -		adr		adw		
		No.	%	No.	%	No.	%	No.	%	No.	%	
HBsAg +	8	11.8	4	50.0	2	25.0	2	25.0	7	87.5	1	12.5
HBsAb +	22	32.4	17	77.3	3	16.7	2	9.1	18	81.8	4	18.2
Total	30	44.2	21	70.0	5	16.7	4	13.3	25	83.3	5	16.7
HBsAg - and HBsAb -	38	55.8	3	8.0	23	60.5	12	31.5	34	89.5	4	10.5
Total	68	100	24	35.3	28	41.2	16	23.5	59	86.8	9	13.2
Cases with acute viral hepatitis	8		8						6	75.0	2	25.0

+ Positive - negative

Table 2 Detection of HBsAg in vaginal discharge of five HBsAg-carrier-state wives

Findings in vaginal discharge		Day of menstrual cycle											
		1		2		3		4		5		6	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
HBsAg+	2	40	3	60	3	60	2	40	2	40	1	20	0

+ Positive

HBsAb 18.1% (Nishioka *et al.*, 1973). By comparison, the present study shows that the incidence of HBsAg and HBsAb in husbands with HBsAg carrier-state wives is extremely high (44.2%). As expected the husbands of the HBsAg-negative women were also HBsAg-negative.

Some authors reported that the radioimmunoassay (RIA) method detected HBsAg in sputum samples (Kawana *et al.*, 1973; Villarejos *et al.*, 1974). In the present study, however, R-PHA failed to detect HBsAg in any of the 68 sputum and 18 cervical mucus samples from premenstrual HBsAg carrier-state women. This fact suggests that the potential for HBsAg infection via sputum or cervical mucus is low. Although HBsAg was not detected in the vaginal discharge of women beyond day 7 of the menstrual cycle, it was detected up to day 6.

We believe, therefore, that our findings support the hypothesis of sexual transmission of HBsAg, especially if intercourse occurs during or immediately after menstruation.

Serum eAb was detected in 23 (60.5%) of 38 carrier-state women whose husbands were serum HBsAg-negative or HBsAb-negative, while only five (16.7%) of 30 women with HBsAg-positive or HBsAb-positive husbands were serum eAb-positive.

Of 30 carrier-state women with husbands infected with HBsAg or HBsAb, 21 (70%) were found to be eAg-positive. Eight (26.7%) of the 30 husbands had a history of acute viral hepatitis shortly after marriage and eAg was detected in the sera of all eight wives.

When the group in which HBsAg transmission had occurred (30 cases, 44.2%) is compared with that in which it had not (38 cases, 55.8%), the serum eAg detection rate among the wives was 8.8 times higher in the transmission-positive group. Furthermore, the serum eAb detection rate among the women was 3.6 times higher in the transmission-negative group.

The results of Okada *et al.* (1976) and of our earlier study (Inaba *et al.*, 1977) on vertical HBsAg transmission suggest that the presence of eAg in maternal sera may be a valuable indicator for predicting the potential for vertical transmission of hepatitis B virus (HBV). From our present findings we consider further that the presence of serum eAg in carrier-state wives may also represent an indicator for predicting the potential for transmission of HBsAg to their husbands. Since eight of 30 HBV-infected husbands had a marital history of acute viral hepatitis and that eAg was detected in the sera of all eight wives we suggest that husbands with eAg-positive wives should be followed up closely to detect hepatitis B at the earliest possible stage.

The present data confirm that transmission of HBsAg may be sexual and suggest that, in Japan, the transmission of HBsAg from carrier-state wives to

their husbands is an important route of HBV infection. Furthermore, our finding that 70% of the wives with HBV-infected husbands were eAg positive suggests that the e-system determination of carrier-state wives may be valuable in predicting the potential of sexual HBV infection.

We wish to thank Ms U. Petralia for her excellent assistance.

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Staphylococcus saprophyticus in the aetiology of nongonococcal urethritis

B. HOVELIUS, I. THELIN, AND P.-A. MÅRDH

From the Department of Medical Microbiology, University of Lund; and the Department of Dermatology and Venereology, University Hospital, Lund, Sweden

SUMMARY The occurrence of *Staphylococcus saprophyticus*, *Chlamydia trachomatis*, and *Neisseria gonorrhoeae* in urethral specimens of 252 men attending a venereal disease clinic was studied. When using a selective broth medium containing novobiocin and nalidixic acid, *Staph. saprophyticus* was isolated from 20.8% of 178 men with symptoms of urethritis and from 14.9% of 74 men without such symptoms. *Staph. saprophyticus* was found significantly less often in controls (7.1% of 56) than in the men with symptoms of urethritis. In the 35 men from whom *Staph. saprophyticus* was recovered more than 10 leucocytes per high power field in urethral smears occurred more often than in those from whom this organism, or either of the other two agents, were not isolated. No differences were found in the symptoms reported by the men harbouring *Staph. saprophyticus* or *C. trachomatis* or those with negative cultures. The results of the present study tend to suggest that *Staph. saprophyticus* is the aetiological agent of some cases of nongonococcal urethritis.

Introduction

In recent years 25-45% of all cases of nongonococcal urethritis (NGU) in men have been attributable to *Chlamydia trachomatis* infection (Holmes *et al.*, 1975; Oriel *et al.*, 1976; Alani *et al.*, 1977; Bowie *et al.*, 1977a; Ripa *et al.*, 1978). Herpes simplex virus, *Candida albicans*, and *Trichomonas vaginalis* are responsible for some (5-10%) cases of NGU (Catterall, 1975; Holmes *et al.*, 1975).

There is also some evidence that *Ureaplasma urealyticum* is the pathogen responsible for some cases of NGU. An association between a primary episode of NGU and *U. urealyticum* has been claimed on the basis of therapeutic studies and quantitative cultures of the organism (Prentice *et al.*, 1976; Bowie *et al.*, 1977a).

Staphylococcus saprophyticus (earlier named *Micrococcus*, subgroup III) is known to be a frequent cause of acute cystourethritis in young adult women (Maskell, 1974; Sellin *et al.*, 1975). *Staph. saprophyticus*, like *Staphylococcus cohnii* and *Staphylococcus xylosus*, differs from other

coagulase-negative staphylococcal species (for example, *Staphylococcus epidermidis*) in being less susceptible to novobiocin (minimum inhibitory concentration $>2.0 \mu\text{g/ml}$).

In the present study, we investigated the occurrence of *Staph. saprophyticus*, *C. trachomatis*, and *Neisseria gonorrhoeae* in urethral specimens of men attending a venereal disease clinic. For comparison male medical students and laboratory staff were also studied. A selective culture medium was developed for the isolation of *Staph. saprophyticus*.

CLINIC POPULATION

Groups 1 and 2

The patients consisted of 252 men between 15 and 54 years of age (median age 25 years), who attended the Department of Dermatology and Venereology, University Hospital, Lund, during two periods, namely, June to August 1977 (96 men) and August to October 1978 (156 men). Of the 252 men, 178 (70.6%) had symptoms suggesting urethritis, that is, urethral discharge, or dysuria, or both (Group 1). The remaining 74 (29.4%) men were symptomless (Group 2). The most common reason for the latter patients attending the clinic was that a sexually transmitted disease had been diagnosed in their sexual partner(s).

Address for reprints: Dr P.-A. Mårdh, Department of Medical Microbiology, University of Lund, Solvegatan 23, S-223 62 Lund, Sweden.

Received for publication 1 March 1979

CONTROLS

Group 3

Fifty-six male medical students and male laboratory staff (20 to 37 years of age, median age 25 years) were studied during the same periods as the men in groups 1 and 2. None of these persons, according to a detailed questionnaire, currently had or had previously had a history of genitourinary disease.

SAMPLING TECHNIQUE AND ISOLATION PROCEDURES

Urethral specimens from the 252 men in groups 1 and 2 were cultured for *Staph. saprophyticus*, *C. trachomatis*, and *N. gonorrhoeae*, while those from the men in group 3 were cultured only for *Staph. saprophyticus*.

Staphylococcus saprophyticus

A urethral specimen was collected with the aid of a cotton-tipped swab, which was first used to inoculate a gonococcal medium, and then transferred to a selective medium (Hovelius and Mårdh, 1977a) of the following composition: tryptone broth (Oxoid), pH 7.2, with the addition of 150 µg nalidixic acid (Winthrop) and 2 µg novobiocin (Sigma) per ml. In 156 of the patients the swab was also used to inoculate blood agar plates before it was transferred to the broth medium. The broth was incubated at 37°C for 18 hours before being seeded on to fresh blood agar plates; these consisted of Blood Agar Base No. 2 (Oxoid) with 4% defibrinated horse blood. The plates were read after 24 hours' incubation at 37°C; from each plate at least six staphylococcal colonies were selected for further studies.

The staphylococci were tested for coagulase production. Coagulase-negative strains were tested by disc diffusion tests (see below) for their susceptibility to novobiocin. Strains producing an inhibitory zone of less than 15 mm in diameter when discs containing 5 µg novobiocin were used were considered to be resistant (Hovelius and Mårdh, 1977b). Such strains were further classified as to species by biochemical tests according to Kloos and Schleifer (1975) and the Subcommittee on Taxonomy of Staphylococci and Micrococci (1976). Furthermore, all strains were also tested on agar plates containing glycerol and erythromycin (Schleifer and Kloos, 1975) to establish that the strains tested were staphylococci and not micrococci.

Neisseria gonorrhoeae

Urethral specimens for the culture of gonococci were collected with a cotton-tipped swab, which, immediately after sampling, was used to inoculate a haematin agar medium (Mårdh *et al.*, 1978). After

inoculation, the plate was incubated at 37°C with regulated CO₂ atmosphere and humidity. The methods used to identify *N. gonorrhoeae* were reported earlier (Mårdh *et al.*, 1978).

Chlamydia trachomatis

Specimens for the culture of chlamydiae were collected by means of a calcium-alginate swab (Inolex Corp., Glenwood, Illinois, USA), which was gently rotated 2-3 cm into the urethra before withdrawal. The swabs were transported in a sucrose-phosphate buffer (Gordon *et al.*, 1969) supplemented with gentamicin 10 µg (Schering), amphotericin B 2.5 µg (Squibb), and vancomycin 100 µg (Upjohn) per ml. The specimens were stored at 4°C if the culture could be started within 24 hours of sampling; otherwise they were stored at -20°C until required. *C. trachomatis* was isolated on cycloheximide-treated McCoy cells as described elsewhere (Ripa and Mårdh, 1977).

MICROSCOPY

Urethral smears from all men in groups 1 and 2 were heat-fixed and stained with methylene blue for the detection of leucocytes and gonococci. The number of leucocytes per high power field (HPF) ($\times 100$ objective) in each smear was graded as follows: <10, 10-30, and >30 (mean of ten HPFs). All specimens were studied under the same microscope by four different physicians.

ANTIBIOTIC SUSCEPTIBILITY TESTS

The 48 strains of *Staph. saprophyticus* isolated from the men in groups 1 and 2 were tested for their susceptibility to penicillin V, cephalixin, nalidixic acid, oxytetracycline, sulphadimidine, and sulphamethoxazole/trimethoprim by the disc diffusion technique (Ericsson and Sherris, 1971) using Wellco susceptibility test agar. The medium and the antibiotic discs were purchased from AB Biodisk (Stockholm, Sweden).

All 48 strains were also tested for β -lactamase production by means of chromogenic cephalosporin (O'Callaghan *et al.*, 1972).

TREATMENT AND FOLLOW-UP

All patients in whom gonorrhoea was diagnosed by microscopy of urethral discharge were treated with pivampicillin 1.4 g orally (Pondocillin^R, Löwens, Denmark) and probenecid 1 g (Probecid^R, Astra, Sweden). Patients in whom microscopical examination of urethral discharge did not indicate gonorrhoea but in whom cultures were nevertheless positive for *N. gonorrhoeae*, whether or not *Staph. saprophyticus* or *C. trachomatis* had been isolated, received the same treatment when re-examined one

week after the first attendance. If *C. trachomatis* had been isolated, whether or not *N. gonorrhoeae* or *Staph. saprophyticus* had been isolated, the patients were prescribed lymecycline 0.3 g (Tetralsal[®], Carlo Erba, Italy) twice daily for one week at the first re-examination. In men who had symptoms of urethritis and from whom *Staph. saprophyticus*—but not *C. trachomatis* or *N. gonorrhoeae*—had been isolated, phenoxymethylpenicillin 0.65 g (Kävepenin[®], Kabi, Sweden) was given three times daily for 10 days.

At the first, as well as at subsequent, follow-up examinations (which were carried out about one week after the first visit and one week after treatment) all culture studies were repeated.

STATISTICAL METHOD

All the statistical calculations were performed using the χ^2 method with Yates's correction. The degree of freedom for each P value given was one.

Results

ISOLATION OF MICRO-ORGANISMS

Staph. saprophyticus was isolated from 37 (20.8%) of the men in group 1, who had symptoms of urethritis. The corresponding figures for the men in groups 2 and 3 without symptoms were 11 (14.9%) and four (7.1%) respectively (Table 1).

Table 1 Culture results for urethral samples from 252 men attending a venereal disease clinic

Organism isolated	No. of patients		
	Symptoms of urethritis (group 1)	Symptomfree (group 2)	Total
<i>Staph. saprophyticus</i>	25	10	35
<i>C. trachomatis</i>	31	9	40
<i>N. gonorrhoeae</i>	27	3	30
<i>Staph. saprophyticus</i> and <i>C. trachomatis</i>	9	1	10
and <i>N. gonorrhoeae</i>	3	0	3
<i>C. trachomatis</i> and <i>N. gonorrhoeae</i>	10	1	11
Total	178	74	252

Staph. saprophyticus was isolated from only six of 156 urethral specimens collected from men in groups 1 and 2 when inoculated directly on to blood agar plates, while the organism was found in 32 of the same specimens when the selective broth medium was used.

Staph. saprophyticus occurred more often in the men in group 1 than in those in group 3 ($P < 0.05$), while the difference between the isolation frequency of *Staph. saprophyticus* in groups 2 and 3 was not significant. This was also true when the isolation frequency in groups 1 and 2 was compared with that in group 3 and when that in groups 1 and 2 was compared.

N. gonorrhoeae was found in 22.5% and *C. trachomatis* in 28.1% of the men in group 1; the

corresponding figures in group 2 were 5.4% and 14.9% for *N. gonorrhoeae* and *C. trachomatis* respectively.

Staph. saprophyticus was isolated from three (6.8%) of the men with gonorrhoea and from 10 (16.4%) of the 61 men harbouring *C. trachomatis* (Table 1).

RELATION OF MICROSCOPY AND CULTURE RESULTS

The culture results of groups 1 and 2 are related to the number of leucocytes per HPF in urethral smears in Table 2. None of the men from whom more than

Table 2 Number of leucocytes per high power field (HPF) ($\times 100$ objective) in urethral smears of male patients (groups 1 and 2) in relation to micro-organisms isolated*

Organism isolated	No. of patients	No. of leucocytes/HPF		
		<10	10-30	>30
<i>Staph. saprophyticus</i>	35	15	11	9
<i>C. trachomatis</i>	40	9	9	22
<i>N. gonorrhoeae</i>	28	2	6	20
Negative results for all three organisms	123	82	21	20

*Men from whom more than one of the three organisms was isolated are not included

one of the three organisms were isolated is included in the Table. *Staph. saprophyticus* ($P < 0.05$), *N. gonorrhoeae* ($P < 0.001$), and *C. trachomatis* ($P < 0.001$) all occurred significantly more often in those men who had more than 10 leucocytes per HPF than in those with fewer. Approximately one-third of the men from whom none of these organisms was recovered also had more than 10 leucocytes per HPF. This was also true for most (20/35) of those infected with *Staph. saprophyticus*.

RELATION OF SYMPTOMS AND CULTURE RESULTS

The symptoms reported by the men in group 1 are related to the culture results in Table 3. Only those

Table 3 Symptoms of men with urethritis (group 1) in relation to micro-organism isolated from urethral specimens*

Organisms isolated	No. of men (group 1) with				
	Discharge		Painful micturition		
	Watery	Purulent	itching	itching	itching
<i>Staph. saprophyticus</i>	(n) 23	5	8	15	3
<i>C. trachomatis</i>	(n) 31	12	11	22	23
<i>N. gonorrhoeae</i>	(n) 27	4	22	18	3
Negative results for all three organisms	(n) 73	19	23	46	19

*Men from whom more than one of the three organisms was isolated are not included

men who harboured one of the three organisms are presented in the Table. Among the men who had symptoms, discharge was reported more often in those with gonorrhoea than in those infected with *C. trachomatis* or *Staph. saprophyticus*. Watery discharge was reported more often by men with chlamydial infection than by those with gonorrhoea, while the converse was true for purulent discharge. There was no significant difference regarding the occurrence of purulent or watery discharge in men from whom *C. trachomatis* and *Staph. saprophyticus* were isolated and from those in whom these organisms or gonococci could not be demonstrated. Dysuria was reported with approximately the same frequency regardless of the result of the culture studies.

ANTIBIOTIC SUSCEPTIBILITY AND CHROMOGENIC CEPHALOSPORIN TESTS

The disc diffusion tests indicated that, except for four strains, *Staph. saprophyticus* was susceptible to all the antibiotics tested, although all strains were resistant to nalidixic acid (Table 4). The strains did not produce β -lactamases according to the results of the chromogenic cephalosporin test.

Table 4 Antibiotic susceptibility of 48 strains of *Staphylococcus saprophyticus* isolated from urethral specimens of men attending a venereal disease clinic (groups 1 and 2)

Antibiotic	Susceptible	Intermediate	Resistant
Penicillin V	48		
Cephalexin	48		
Nalidixic acid	0		48
Oxytetracycline	45		3
Sulphadimidine	44	2	2
Sulphamethoxazole/ trimethoprim	48		

TREATMENT

In 12 of the 16 men in group 1 harbouring *Staph. saprophyticus* and who returned for re-examination, this organism could not be isolated, irrespective of whether phenoxymethylpenicillin, lymecycline, or pivampicillin had been given. All 16 men had become symptomfree. In one of the four men with persistent growth of *Staph. saprophyticus*, a renal calculus was later diagnosed. In another four men (one from group 1 and three from group 2) harbouring *Staph. saprophyticus*, and who had not been given antibiotics, the organism could no longer be isolated from urethral specimens at re-examination two weeks after the initial examination.

None of the 44 men in groups 1 and 2 with gonorrhoea was culture-positive for *N. gonorrhoeae* after treatment and 42 were symptomfree. Thirty-two of the 61 chlamydia-positive men returned for

follow up. All of them had become culture-negative for *C. trachomatis* and 29 were symptomfree.

Discussion

Staph. saprophyticus is a common cause of acute, often haemorrhagic, cystourethritis in young adult women (Maskell 1974; Sellin *et al.*, 1975). Urinary tract infections with *Staph. saprophyticus* in women occur most frequently in those age groups in which the highest incidence of sexually transmitted genital infections occur. A seasonal variation in the incidence of such infections has been noted; the highest incidence occurs in early autumn (Telander and Wallmark, 1975; Maskell and Pead, 1977; Hovelius and Mårdh, 1978), that is during the same period of the year as the present study was undertaken. A similar variation can also be shown for most sexually transmitted infections (Wright and Judson, 1978), but this cannot be demonstrated for urinary tract infections caused by *Escherichia coli* (Maskell and Pead, 1977).

In men, cystitis with *Staph. saprophyticus* most frequently occurs in men over 60 years of age with benign hyperplasia of the prostate. However, we have observed acute cystitis caused by *Staph. saprophyticus* in young adult men without obstructive urinary tract disease (Hovelius and Mårdh, 1978). Some of these men had attended a venereal disease clinic because they suspected that they had contracted a venereal infection.

Staph. saprophyticus is known to occur as a pathogen solely in the urinary tract. In clinical microbiological laboratories, *Staph. saprophyticus* is seldom recovered from specimens other than urine, in which it rarely seems to occur as a contaminant. *Staph. saprophyticus* may be isolated from the skin of the arms and legs, although transiently and in low numbers (Kloos and Musselwhite, 1975). With a selective culture medium, Pead and Maskell (1977) isolated *Micrococcus* subgroup III from rectal swabs from 10 of 156 women.

It has been argued that the capacity of a bacterium to adhere to epithelial cells is a prerequisite for its ability to cause mucosal infections (Gibbons, 1977). In *in vitro* experimental studies on bacterial adhesion to urothelial cells, we found *Staph. saprophyticus* to adhere in significantly greater numbers to such cells than to epithelial cells from other sites, for example, buccal and skin cells (Colleen *et al.*, in press). Among other genital and urinary tract pathogens only freshly isolated strains of *N. gonorrhoeae*, producing T1 and T2 colonies, adhered in greater numbers per urothelial cell than did *Staph. saprophyticus*. This pronounced capacity of *Staph. saprophyticus* to

adhere to urothelial cells may explain the organism's tropism for the urinary tract (Mårdh *et al.*, 1979).

When urethral specimens are cultured on blood agar, *Staph. saprophyticus* is seldom isolated, as shown in the present study. Bowie *et al.* (1977b) failed to isolate this organism from any of 62 patients with NGU. The poor growth of *Staph. saprophyticus* on blood agar plates inoculated with urethral specimens might be because this bacterium occurs in low numbers or because it is inhibited by microbial interaction. Thus, we have found that strains of *Staph. epidermidis* may inhibit growth of *Staph. saprophyticus* on artificial culture media (unpublished data).

When our selective culture medium was used, *Staph. saprophyticus* could be isolated from the urethral specimens of men without either a history or symptoms of urethritis or urinary tract infection. Thus, the organism was recovered from 7.1% of male students and laboratory staff.

The role of *Staph. saprophyticus* in NGU is difficult to establish, as is that of other organisms which may occur in the normal flora, namely *U. urealyticum*. *Staph. saprophyticus* was isolated significantly more often from the men with symptoms of urethritis (group 1) than from medical students and laboratory staff (group 3). The organism was found significantly more often among the men in groups 1 and 2 with an increased number of leucocytes in their urethral smears than in those men in these groups with less than 10 leucocytes per HPF. It is notable that in the patients with gonorrhoea the frequency of isolation of *Staph. saprophyticus* was as low as in the men in group 3.

Staph. saprophyticus was susceptible *in vitro* to all antibiotics tested, including a number of drugs commonly prescribed to men with genital and urinary tract infections. In this respect, however, nalidixic acid was an exception (Hovelius and Mårdh, 1977b). In a previous study this antibiotic constantly failed to cure patients with urinary infection caused by *Staph. saprophyticus* (Hovelius *et al.*, 1979). Of the 16 men studied who harboured *Staph. saprophyticus*, and who were treated with penicillin, pivampicillin or lymecycline, 12 were culture-negative and symptomfree at follow up. Three men still harboured *Staph. saprophyticus* but had no symptoms and less than 10 leucocytes per HPF in urethral smears at re-examination. In one man, in whom *Staph. saprophyticus* persisted despite antibiotic treatment, a renal calculus was diagnosed. This observation agrees with our earlier findings (Hovelius *et al.*, 1979). We have occasionally diagnosed persistent bacteriuria with *Staph. saprophyticus* which proved intractable to repeated courses of antibiotics to which the organism was

susceptible *in vitro*. These men became culture-negative after the removal of the calculus.

The finding that *Staph. saprophyticus* and symptoms of urethritis had disappeared within a fortnight in some men not given antibiotic treatment agrees with the observation of spontaneous cure in women of urinary tract infections caused by coagulase-negative staphylococci (Mabeck, 1969).

In conclusion, the result of the present study suggests that *Staph. saprophyticus* should be considered as a potential aetiological agent of some cases of NGU in men.

This study was supported by grant No. 16X-4509 from the Swedish Medical Research Council.

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Epidemic of amoebiasis and giardiasis in a biased population

B. H. KEAN,* DANIEL C. WILLIAM,† AND STEVEN K. LUMINAIS*

From the *Tropical Medicine Unit, Department of Medicine, New York Hospital-Cornell Medical Center, and the Gay Men's Health Project†

SUMMARY Of 126 homosexual men in a selected population in New York City, 31.7% were infected with *Entamoeba histolytica*, 18.3% with *Giardia lamblia*, and 39.7% with one or both on a single stool examination. Of 5885 clinic and hospital patients examined in the same laboratory by the same methods, 1.3% were infected with *E. histolytica*, 2.1% with *G. lamblia*, and 3.3% with one or both. Evidence indicates that an epidemic of intestinal protozoan infection exists in the homosexual male population in New York City. The difficulty in making a diagnosis, inadequate therapy, failure to alert potential victims, and official neglect of the epidemic have combined to create a dangerous situation.

Introduction

The purpose of this paper is to establish, by a brief historical survey and evidence provided by a prospective study, that thousands of individuals in New York City are presently infected with *Entamoeba histolytica* or *Giardia lamblia* or both. The notion that an epidemic of amoebiasis and giardiasis exists in a large metropolitan area may seem incredible. Evidence has accumulated, however, indicating that part of the sexually active adult community in New York City is heavily infected with *E. histolytica* and *G. lamblia*. The first published report of the relationship between such protozoan infections and sexual behaviour was made by Most (1968). He described four cases of amoebiasis in homosexual men in New York City linked only by shared sexual experience. Some years previously, one of us (B.H.K.) had asked the New York City Department of Health to investigate the water supply in certain areas of Greenwich Village, because it seemed that more cases of amoebiasis came from that part of the city than from elsewhere. The water supply was found to be uncontaminated.

During the next decade, little attention was paid to the problem. A spate of articles then appeared suggesting that in New York City and elsewhere the incidence of amoebiasis and giardiasis in the sexually active homosexual population was high and

increasing. Kean (1976a), William *et al.* (1977), Kazal *et al.* (1976), Meyers *et al.* (1977), Sohn and Robilotti (1977), Schmerin *et al.* (1977), Mildvan *et al.* (1977a), Hurwitz and Owen (1978), and others reported on this association. Simultaneously, evidence accumulated that viruses—for example, hepatitis B, and bacterial pathogens, such as *Shigella* spp, *Salmonella* spp, and some helminths, such as *Enterobius vermicularis*—can be transmitted during sexual practices (Drusin *et al.*, 1976; Bader *et al.*, 1977; Dritz *et al.*, 1977; Mildvan *et al.*, 1977b). Frequent sexual exposure involving fellatio and anilingus with multiple partners is obviously the mechanism of transmission of enteric pathogens. Such a pattern of sexual activity occurs throughout the sexual spectrum in both homosexuals and heterosexuals but is probably more common among the former.

Our project was designed to determine how heavily infected with *E. histolytica*, *G. lamblia*, *Salmonella* spp, and *Shigella* spp sexually active homosexual men in New York City were, fully recognising that this group represented only a single, small segment of the homosexual and heterosexual populations at risk.

Patients and methods

The study population consisted of volunteer patients presenting to the Gay Men's Health Project, which is primarily a screening facility for the detection of syphilis and gonorrhoea in sexually active homosexual men. The clinic is located in Greenwich Village and provides services to patients residing in

Address for reprints: Dr B. H. Kean, Cornell University Medical College, 1300 York Avenue, New York, NY 10021, USA

Received for publication 20 November 1978

greater metropolitan New York. The clinic population is predominantly young adult, college-educated, middle-class, white men. Most of the patients are very active sexually and present periodically to the clinic for routine screening for venereal disease.

All patients were informed of the nature of this study by printed information sheets and by direct counselling by the clinic staff and were invited to participate. Those with symptoms suggesting enteric disease were urged to participate.

Each patient was asked to bring a morning stool specimen to the parasitology laboratory of the New York Hospital-Cornell Medical Center. He was asked whether he had symptoms, whether he had ever had amoebiasis, giardiasis, or severe diarrhoeal illness, and whether he had taken any antibiotics or other drugs for the treatment of intestinal infection during the preceding three weeks. No patient was excluded from the study for any reason. A total of 126 patients participated.

Each specimen was examined microscopically both directly and, after concentration, by a modified Ritchie (1948) technique. The concentrate was stained with either iodine or merthiolate-iodine-formalin stain. Two-thirds of the specimens were cultured for protozoa using a standard culture (Cleveland and Collier, 1930). The first 63 specimens were cultured for *Salmonella* spp and *Shigella* spp in the microbiology laboratory of the New York Hospital-Cornell Medical Center by standard bacteriological techniques routinely used for the isolation and identification of these organisms. Two aliquots from each of the first 100 specimens were preserved in 6% formalin and MIF solution for future reference.

Results

CLINICAL

Approximately three-quarters of the patients reported gastrointestinal symptoms of some sort; almost always these were mild. Complaints included flatulence, cramp, diarrhoea, mucus or blood in the faeces, pain, constipation, fatigue, sweating, bleeding gums, upset stomach, and nausea. Six patients had recently taken antibiotics.

PARASITOLOGICAL

Results of the faecal examinations are given in the Table. The results of 5885 consecutive stool examinations performed in the same laboratory by the same techniques and by the same technicians are given for comparison. This population, also biased, represented hospital inpatients and outpatients of the New York Hospital whose physicians considered a

Table Results of parasitological examination of stool specimens from two biased population groups

Protozoa isolated	Male homosexual community (1977)		Inpatients and outpatients of the New York Hospital (1976-77)	
	No.	%	No.	%
One or more	61	48.4	305	5.2
<i>Entamoeba histolytica</i>	39	31.7	74	1.3
<i>Giardia lamblia</i>	23	18.3	123	2.1
<i>Entamoeba coli</i>	15	11.9	82	1.4
<i>Endolimax nana</i>	19	15.1	84	1.4
<i>Iodamoeba butschlii</i>	4	3.2	13	0.2
<i>E. histolytica</i> and <i>G. lamblia</i>	12	9.5	5	0.08
<i>E. histolytica</i> or <i>G. lamblia</i> or both	50	39.7	192	3.3
Total no. of specimens	126		5885	

parasitological examination of the stool necessary for a number of different reasons.

Thirty-one (24.6%) patients had a previous diagnosis of amoebiasis and one of giardiasis. Of these 31 patients, 18 (58.1%) had negative results in this study: five were infected with *E. histolytica*, two with *Endolimax nana*; two with *E. coli*; one with *E. histolytica* and *E. coli*; one with *E. histolytica* and *G. lamblia*; one with *E. histolytica* and *E. nana* and one with *G. lamblia*. Thus, since 58% of these previously infected patients had negative results compared with 51.6% of all the patients in the study, and compared with 49.5% of the patients with no previous history, this group did not artificially increase the 'yield' of our study.

BACTERIOLOGICAL

Two of the first 63 (3.2%) specimens gave positive results for *Shigella*: one for *S. sonnei* and one for *S. flexneri* type 3. No *Salmonella* spp were cultured.

Discussion

Sawitz and Faust (1942) pointed out many years ago that a single stool examination yields only a small percentage of the positive diagnoses that would be made if five stools from the same individuals were examined. Their figures (Sawitz and Faust, 1942) suggested that only one-third of potentially positive results were found on one stool examination. Our own experience indicates that in the presence of symptoms that figure may be low. If the stools were examined as soon as they were passed or if a saline cathartic had been used and several specimens were examined in sequence, the figure of 39% for one or more protozoan pathogens certainly would have been higher. Thus, the figure of 39% could be increased easily to at least 50% and possibly to 65% with a

more intensive coprological study of the same individuals. We will assume, therefore, that at least half of our study group was infected.

The study group represented a biased population. These results must not be extended to the entire homosexual population, to the heterosexual population, or to other communities. Nevertheless, cautious interpretation of the data leads to some interesting conclusions. New York City has a population of about eight million people. The population at risk for venereal amoebiasis and giardiasis is a sub-group of the adult population who engage in oral/anal sexual contact and have numerous sexual partners. One large segment of this population has been identified as New York City's homosexual male community (Schmerin *et al.*, 1978), but the total size of this community is unknown. We do know, however, that 10% of men are more or less exclusively homosexual during the three years between the ages of 16 and 35, and 4% of white men are exclusively homosexual throughout their adult lives (Kinsey *et al.*, 1948). New York City has more than 75 homosexual bars and bath-houses (Gay Yellow Pages, 1979) suggesting a minimum supporting population of more than 75 000. The 10 000 or more clients of the Gay Men's Health Project would, therefore, represent only a small proportion of the city's total sexually active homosexual male community (10 000 has been the mean annual attendance between 1972 and 1978).

If about 50% of our biased population was infected with amoebiasis or giardiasis, how many of New York City's population are also infected? There are too many variables and too many assumptions to allow a statistical estimate, but several non-scientific mathematical manipulations suggest numbers ranging from 10 000 to 50 000. Many of those infected escape detection at treatment centres. Large numbers remain asymptomatic. Similarly, few of the asymptomatic cyst carriers are detected because of the difficulty of accurate diagnosis. All of these untreated cases add to the expanding reservoir of infection, allowing further transmission from person to person.

Coprology remains a neglected science. The proper examination of the stool is requested by few physicians and accomplished by fewer laboratories. The accurate diagnosis of *E. histolytica* infection demands careful microscopical study by an expert, the use of special concentration methods, staining, and culture. It is expensive and time-consuming. Leucocytes are mistaken for *E. histolytica* even by experts who adopt cultural and staining methods that are rarely used routinely. Krogstad *et al.* (1978) emphasised the incidence of overdiagnosis (false-positive results) and underdiagnosis (false-negative

results), and discussed in detail the problems of accurate diagnosis.

Our knowledge of amoebiasis and giardiasis has been increased recently by the reports of Krogstad *et al.* (1978) and Wolfe (1978) respectively. Although about six drugs are being used for the treatment of intestinal amoebiasis, there is no unanimity regarding either the preparation or the dosage (Kean, 1976b).

RECOMMENDATIONS

The public health authorities—federal, state, and city—who have so far shown only token interest in this subject must intervene. A newsletter from the Commissioner of Health to practising physicians (*Sexually Transmitted Diseases Newsletter*, 1977) and the mild intellectual interest of the infectious disease divisions of government agencies is hardly adequate to meet the needs. When, in 1933, following the World's Fair, an epidemic of amoebiasis occurred which affected 1500 people with several deaths in different parts of the country, the United States Public Health Service mobilised a team, engaged special consultants, alerted all health departments, and acted in a responsible manner (Bundesen *et al.*, 1933; McCoy, 1936). The epidemic in New York City is vastly greater in numbers and in potential for disaster. What the situation is in other cities, with the exceptions of San Francisco and Seattle, is not known.

Clearly, there is a serious need for a national assessment of this problem. The scope of sexually acquired enteric protozoan infection must be fully defined; only then can resources be intelligently allocated for the educational, diagnostic, and treatment facilities necessary to control and perhaps eliminate these 'newest' of old diseases.

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Correspondence

TO THE EDITOR, *British Journal of Venereal Diseases*

Treatment of lymphogranuloma venereum with rifampicin

Sir,
Ridgway *et al.* (1978) tested the effect of 22 antimicrobial agents *in vitro* against SA2f, a laboratory maintained strain of *Chlamydia trachomatis*, which is immunologically identical with the LGVII serotype (Wang and Grayston 1970). Rifampicin was found to be the most active of the drugs tested. This laboratory finding has not been evaluated clinically. Presentation of results of his work (Ridgway, 1976) at an international conference on STD stimulated us to study the efficacy of rifampicin in the treatment of patients with lymphogranuloma venereum (LGV).

We studied eight patients, who were seen in 1976 and 1977 at the outpatient department of the dermatological service in Surinam. All showed inguinal lymphadenopathy, typical for LGV, and six of them also showed a small non-indurated ulcer on the genitals. The Venereal Disease Research Laboratory (VDRL) test gave negative results in all cases, and the six patients with ulcers showed no treponemes by darkfield microscopy and no organisms suggesting *Haemophilus ducreyi* or *Donovania granulomatis* by Gram-stained and Giemsa-stained smears. Clinical diagnosis of LGV was made in all cases.

The presence of type-specific antibodies against *C. trachomatis* in the sera was examined by the microimmunofluorescence (MIF) test as described by Wang *et al.* (1975). The antigens used were of the three serotypes of *C. trachomatis* of the LGV type, LGVI, LGVII, and LGVIII. The MIF was performed with FITC labelled polyvalent conjugates. Following Wang, only reactions with a titre 1/8 were considered as positive. The Frei test was performed in four cases.

The results of the MIF and the Frei tests are presented in the Table. The clinical diagnosis of LGV was supported by the results of the MIF test in all cases.

The patients were treated with oral rifampicin 600 mg every morning until symptoms had disappeared; this occurred after two weeks in seven patients and after three weeks in one. No side effects of the

Table Results of microimmunofluorescent (MIF) antibody and Frei tests before start of treatment.

Case No.	Age Sex	Frei test results	Reciprocal MIF antibody titres		
			LGVI	LGVII	LGVIII
1	F 17	—	256	256	—
2	M 24	ND	—	>64	—*
3	M 20	ND	—	>64	16
4	M 24	ND	32	128	64
5	F 42	ND	16	64	16†
6	M 35	+	—	32	—
7	M 22	+	—	32	—
8	F 23	—	—	64	—

+ Positive — negative

*Two weeks after start of treatment

†Twelve weeks after start of treatment

ND not done

drug were noticed and no relapse of symptoms was observed during a control period of three months.

Rifampicin is highly effective against chlamydiae *in vitro* (Ridgway, 1978). Our results indicate that the drug is effective against LGV clinically. However, we do not recommend rifampicin as drug of choice for the treatment of LGV for the following reasons:

- (1) Most cases of LGV still respond well to treatment with tetracycline, which is a safe drug.
- (2) Indiscriminate use of rifampicin could make this drug less valuable for the treatment of mycobacterial infections, because of the possibility of these organisms developing resistance to it.
- (3) Keshishyan *et al.* (1973) showed that rifampicin-resistant chlamydiae emerged rapidly *in vitro* during egg passage in the presence of the drug.
- (4) Rifampicin is not effective against *Treponema pallidum*. Some clinicians might consider this to be a disadvantage, especially in cases in which the diagnosis of syphilis is not completely excluded, although others would consider it an advantage.

Yours faithfully,

H. E. Menke

J. L. Schuller

E. Stolz

Department of Dermatology,
University Hospital,
Rotterdam

P. L. A. Niemel

Dermatological Service,
Paramaribo,
Surinam

M. F. Michel

Department of Clinical Microbiology,
Erasmus University,
Rotterdam

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TO THE EDITOR, *British Journal of Venereal Diseases*

Preparation of *T. pallidum* extracts from infected rabbit testes

Sir,
When extracting *Treponema pallidum* from infected rabbit testes, either for experimental use or for passage into another rabbit, it is important to maintain optimum survival of the treponemes. Whereas some research groups use a crude testicular extract, others use centrifugation, or centrifugation followed by filtration, to purify the treponemal suspension by removing testicular debris, including spermatozoa.

The effect of these procedures on this apparently delicate bacterium has not been reported to date, although the procedures are widely used. We wish to report their effect on the *in-vitro* retention of motility and virulence of *T. pallidum*.

Treponemes were eluted from the testis into prerduced maintenance medium (slightly modified from Graves *et al.*, 1975) at a concentration of approximately

Table Effect of centrifugation and filtration on the retention of virulence of *Treponema pallidum* (Nichols strain)

Incubation period (hours) before inoculation	No centrifugation/no filtration		Centrifugation only		Centrifugation/filtration (Millipore)		Centrifugation/filtration (Nucleopore)		Centrifugation/filtration (Millipore and Nucleopore)	
	A (days)	B (mm)	A (days)	B (mm)	A (days)	B (mm)	A (days)	B (mm)	A (days)	B (mm)
0	7.5	ND	9.5	ND	7.7	ND	8.7	ND	9.5	ND
24	11.5 (2/4)	3.5 (2/4)	9.5	8.8	13	9.8	11.7	14	11	14.5
48	16 (3/4)	3 (3/4)	14.7	6.5	14.7	6.7	13.7	10.8	13.7	12

A = time period (days) between intradermal inoculation of *T. pallidum* into rabbit and first appearance of syphilitic lesion. The smaller the value, the more readily the *T. pallidum* has grown *in vivo* and, by implication, the better its survival *in vitro*. The number in parenthesis represents the fraction of inoculation sites that developed syphilitic lesions; a value of <4/4 means that some inoculations were unsuccessful; the absence of a value means all four inoculations were successful, the value reported being the mean of four lesions.

B = the mean size of four lesions, except where fewer lesions developed (in parenthesis), measured 33 days after inoculation as the diameter of induration. ND = not done

10⁷/ml, centrifuged at 55×g for five minutes, and filtered through a prefilter (Millipore AP2002500) or a 0.8-µm polycarbonate membrane (Nucleopore) or both. The final filtrate was examined microscopically and appeared to contain no host cells. All *T. pallidum* samples for testing were diluted into prereduced maintenance medium (1/20), incubated anaerobically at 34°C, and examined microscopically over a 48-hour period to determine the percentage motility of the treponemal suspension. Samples (0.5 ml) were taken at 0, 24, and 48 hours and diluted 1/1 with 20% glycerol in physiological saline. These were stored at -70°C until the completion of the experiment and then inoculated (in quadruplicate) into the shaved backs of rabbits (0.1 ml/site). A different rabbit was used for samples taken at 0, 24, and 48

hours. Rabbits were examined daily for up to one month and the first day of appearance of an indurated lesion was recorded.

No difference in motility retention was detected in any sample over the 48-hour period. However, other variables measured (that is, number of inoculation sites developing into syphilitic lesions, latent period of infection, and size of syphilitic lesions) all showed that the centrifuged and filtered extracts were in no way inferior to the crude untreated extract (Table). In fact, the extracts centrifuged and filtered through a Nucleopore filter, 0.8 µm with or without a Millipore prefilter, were apparently superior to the crude extract with respect to virulence. Latent periods of infection were shorter and lesion sizes larger.

In conclusion, centrifugation and

filtration of the crude rabbit testicular syphiloma extract appears to enhance the retention of virulence of *T. pallidum in vitro*, presumably by the removal of testicular debris that is deleterious to treponemal survival.

We wish to thank Ian McLean for his technical assistance.

Yours faithfully,

F. Trewartha and S. Graves

Department of Microbiology,
Monash Medical School,
Prahran,
Victoria,
Australia 3181

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TO THE EDITOR, *British Journal of Venereal Diseases*

Campylobacter jejuni in a male homosexual

Sir,
Following the recent letter by Simmons and Tabaqchali (1979), we should like to describe the isolation of *Campylobacter jejuni* from a male homosexual.

A 36-year-old man presented in July 1978 at the Seaman's Dispensary, Liverpool, having noticed pus around the faeces for the preceding three weeks but no diarrhoea. He last had rectal intercourse in January of that year. The only significant clinical finding was slight congestion of the rectal mucosa.

Neisseria gonorrhoeae was neither seen on Gram-stained rectal smear nor isolated

from rectal culture. However, *C. jejuni* was isolated on selective medium from the faeces using a similar technique to that described by Simmons and Tabaqchali (1979). Further smears and culture for *N. gonorrhoeae* four days later gave negative results.

In view of the clinical signs the patient was treated with oral erythromycin 250 mg six hourly for one week. After that time the patient was seen again, and he informed us that the faeces had returned to normal. On proctoscopy there was marked improvement of the rectal congestion. Faecal specimens at this time, and all subsequent specimens, failed to yield *C. jejuni*. After a further week the rectal mucosa was healthy.

This case further emphasises the need to examine specimens of faeces for enteric

pathogens from any patient presenting with abnormal bowel symptoms.

Yours faithfully,

P. B. Carey

Seaman's Dispensary,
Cleveland Square,
Paradise Street,
Liverpool L1 5BH

E. P. Wright

Public Health Laboratory,
Fazakerley Hospital,
Liverpool L9 7AL

Reference

- Simmons, P. D., and Tabaqchali, S. (1979). *Campylobacter* species in male homosexuals. *British Journal of Venereal Diseases*, 55, 66.

Book reviews

Sexually Transmitted Diseases. By C. B. S. Schofield, 1979. Pp. 250. Churchill Livingstone: Edinburgh (£3.25).

The author is certainly not letting the grass grow under his feet and is to be commended on producing a third edition of his popular paperback first published in 1972. The symbolic representations of men predominating over women on the new cover highlights his intention to focus attention on the possibility of the sexually transmitted diseases (STD) manifesting themselves at sites other than the genitals. Thus there are new sections on Australia antigen-positive hepatitis, cytomegalovirus, infectious mononucleosis, and the enteric diseases. The chapters on gonorrhoea have been updated as befits the most common of the venereal diseases still having the greatest research potential.

There are still the distinctive chapters on the medico-social background and management of STDs. Information on the tropical diseases affecting the genitals is also welcome, even to those of us working in the commercial centres of landlocked regions. Chlamydial infections are included where relevant, but I wonder whether the time has now come to devote more space to this important and expanding subject. The references have been revised and extended.

The new enlarged edition, which can still be slipped into the pocket, has improved

spacing and a more attractive format. What it lacks most of all are some pictures or illustrations of the many conditions described. Its remarkably low price should not, however, deter the enthusiast from investing in *Wisdom's Colour Atlas of Venereology* to complement this wide-ranging and informative textbook.

A. L. Hilton*

*Dr Hilton died recently

Handbook on Sexually Transmitted Diseases. By the National Health and Medicine Research Council, 1978. Pp. 64. Australian Government Publishing Service: Canberra (\$A 2.00).

This handbook was produced by the *ad hoc* committee on venereal diseases of the National Health and Medical Research Council in response to a need to give Australian doctors, students, nurses, and other health workers an understanding of sexually transmitted disease. It was not designed as a textbook but does list books for further reading. It, therefore, excludes many details and contentious topics; notwithstanding, it is an excellent, well-produced handbook, which is a pleasure to read.

Careful attention to page layout and sub-headings make for easy referral to any subject, and there is an adequate index (but why have 'introduction' included — look at the front of the book for that, not the index).

All normal sexually transmitted infections are included as well as a section on 'other conditions', which includes such important subjects as 'normal genital structures', 'injuries', and 'skin disease'. The section on Donovanosis is rather disappointing in view of the incidence of disease in nearby Papua New Guinea. Many would not agree that 'a patient can be reassured that a female partner that they may have infected (with non-specific urethritis) will not suffer serious consequences.' This statement spoils an otherwise excellent chapter on the pitfalls of NSU.

Treatment schedules are conveniently grouped in an appendix and are generally adequate. Oral penicillins are specifically stated to be not reliable for the treatment of syphilis, and several similar negative statements are made. It is deplorable that procaine penicillin treatment for gonorrhoea should be spread over two days.

Such a handbook as this would fulfil a need in the United Kingdom as well as in Australia.

P. D. Simmons

Abstracts

These selected abstracts and titles from the world literature are arranged in the following sections:

Syphilis and other treponematoses

(Clinical and therapy; serology and biological false-positive phenomenon; pathology and experimental)
Gonorrhoea

(Clinical; microbiology; therapy)

Non-specific genital infection

Reiter's disease

Trichomoniasis

Candidosis

Genital herpes

Other sexually transmitted diseases

Public health and social aspects

Miscellaneous

Syphilis and other treponematoses (Clinical and therapy)

Yaws and syphilis (letter)

J. JEFFERISS (1979). *British Medical Journal*, 1, 1282

Yaws and syphilis (letter)

A. L. PAHOR (1979). *British Medical Journal*, 1, 1282

Syphilitic hearing loss (editorial)

R. ROTHENBERG (1979). *Southern Medical Journal*, 72, 118-120

Syphilis (Serology and biological false-positive phenomenon)

Problems affecting performance of the fluorescent treponemal antibody absorption test for syphilis

E. F. HUNTER, M. R. ADAMS, L. H. ORRISON, B. J. PENDER, AND S. A. LARSEN (1979). *Journal of Clinical Microbiology*, 9, 163-166

No qualitative differences were found in IgG FTA-ABS tests on 92 stored and 66 fresh sera examined without heating or after inactivation at 56°C for 30 minutes. IgG FTA titres on heated and unheated sera were also similar and it is recommended that the requirement for inactivating sera for the IgG FTA-ABS test should be discontinued.

In quantitative IgM FTA tests inhibition of staining at the lower serum dilutions was

seen in 20 of 22 unheated sera from patients with no history of rheumatoid arthritis, 17 of whom had syphilis. No such prozones were seen with unheated sera from six syphilitic and three non-syphilitic patients, all of whom had rheumatoid arthritis.

When multispot slides are used for FTA tests, mixing of sera from adjacent spots or contamination of slides with treponemes which have become detached during the washing processes may occur and give misleading results. It is recommended that both reactive and non-reactive slides should be examined under visible light to allow a correct assessment of results.

A. E. Wilkinson

Clinical evaluation of a new screening test for syphilis

J. D. DYCHMAN, B. GATENBEIN, R. D. WENDE, AND R. P. WILLIAMS (1978). *American Journal of Clinical Pathology*, 70, 918-921

The Syphla-Chek (SC) test is a screening test carried out on disposable plastic cards with a lipid antigen. Its performance was compared with that of the VDRL and RPR tests on 200 sera from persons presumed to be healthy (premarital tests), sera from patients with untreated primary (80), secondary (29), and latent (32) syphilis and on 146 sera from patients with treated syphilis.

None of the tests was reactive with the normal sera but 35 gave granular reactions in the SC test; one of these gave a borderline and one a reactive FTA-ABS result, but neither patient had a history of syphilis. The SC test was slightly more sensitive than the VDRL in the sera from patients with untreated primary or treated syphilis but the VDRL was more sensitive than the SC test in the small group with

untreated latent infections. The RPR was intermediate in sensitivity between the other two tests. In quantitative tests the titres agreed within one doubling dilution in 90.4% for the VDRL and RPR, 98% for the VDRL and SC, and 98.2% for the RPR and SC. The SC test is thought to be equivalent to the others for screening purposes.

A. E. Wilkinson

False-positive reactions in the rapid plasma reagin-card, fluorescent treponemal antibody-absorbed, and hemagglutination treponemal syphilis serology tests

C. R. PETER, M. A. THOMPSON, AND D. L. WILSON (1979). *Journal of Clinical Microbiology*, 9, 369-372

Syphilis (Pathology and experimental)

Glucose incorporation by *Treponema pallidum*

J. T. BARBIERI AND C. D. COX (1979). *Infection and Immunity*, 24, 291-293

Comparative behaviour of virulent strains of *Treponema pallidum* and *Treponema pertenue* in gradient cultures of various mammalian cells

A. H. FIELDSTEEL, J. G. STOUT, AND F. A. BECKER (1979). *Infection and Immunity*, 24, 337-345

Surface mucopolysaccharides of *Treponema pallidum*

T. J. FITZGERALD AND R. C. JOHNSON (1979). *Infection and Immunity*, 24, 244-251

Relationship of *Treponema pallidum* to acidic mucopolysaccharides

T. J. FITZGERALD, R. C. JOHNSON, AND D. M. FITZ (1979). *Infection and Immunity*, **24**, 252-260

Mucopolysaccharides of *Treponema pallidum*

T. J. FITZGERALD AND R. C. JOHNSON (1979). *Infection and Immunity*, **24**, 261-268

Gonorrhoea (Clinical)**Laboratory-acquired gonococcal conjunctivitis**

S. C. BRUNS AND R. R. TIGHT (1979). *Journal of the American Medical Association*, **241**, 274

Rheumatic fever and gonococcal pharyngitis in an adult

S. CHERIAN, M. R. TABATABAI, AND W. A. CUMMINGS (1979). *Southern Medical Journal*, **72**, 319-321

The arthritis of rheumatic fever in adults may mimic acute gonococcal arthritis. To further characterise this clinical picture the features of six patients presenting with the migratory polyarthritis of acute rheumatic fever have been analysed. Of two men and four women, age range 18-43 years, in five the initial clinical impression was gonococcal arthritis. In all patients, however, the diagnosis of acute rheumatic fever was subsequently established. Characteristics of acute rheumatic fever not commonly described included tenosynovitis in all six and an erythematous rash in three. In three patients synovial fluid WBC exceeded $28 \times 10^9/l$, with more than 90% neutrophils. In five of the six patients gonococcal arthritis was ruled out by appropriate studies and by failure to respond to antibiotics in all patients. The one patient with serum antigonococcal antibodies had gonococcal pharyngitis and acute rheumatic fever.

Authors' summary

Gonorrhoea (Microbiology)**Hen fluorescein-labeled gonococcal lipopolysaccharide antibody in the delayed fluorescent antibody technique for the confirmation of *Neisseria gonorrhoeae***

F. E. ASHTON, R. A. LEITCH, M. B. PERRY, R. WALLACE, AND B. B. DIENA (1979). *Journal of Clinical Microbiology*, **9**, 323-328

A fluorescent antibody reagent (termed anti-LPS conjugate) was prepared from sera obtained from hens immunised with gonococcal R-type lipopolysaccharide. The reagent was absorbed with formalin-treated cells of *Neisseria meningitidis*. The anti-LPS conjugate gave uniform brilliant staining of *Neisseria gonorrhoeae* with little background fluorescence, thus making interpretation and reading of fluorescence simple. The conjugate did not significantly stain cultures of *N. meningitidis*, *Neisseria lactamica*, non-pathogenic *Neisseria* species, or other Gram-negative bacteria. Several preparations of the conjugate provided the same specificity and reproducibility of staining. The anti-LPS conjugate was compared with Difco Laboratories fluorescent antibody conjugate for staining of *N. gonorrhoeae*. Both conjugates stained cells of the light and dark variants of gonococcal colonial types 1 and 2, as well as cells of colonial types 3 and 4. When used for the confirmation of *N. gonorrhoeae*, the anti-LPS and Difco conjugates stained 426 (98.8%) of 431 and 210 (98.6%) of 213 of the gonococcal cultures respectively. Absorption of the anti-LPS conjugate with R-type lipopolysaccharide removed the staining of gonococci. However, absorption of Difco conjugate with R-type lipopolysaccharide did not remove the staining of gonococci, suggesting that the most of the fluorescein-labelled antibody present in the Difco conjugate is directed to gonococcal cell surface components other than lipopolysaccharide. The results of this study indicate that fluorescein-labelled gonococcal lipopolysaccharide antibody should be a reliable fluorescent antibody reagent for the confirmation of *N. gonorrhoeae*.

Authors' summary

Identification of *Neisseria gonorrhoeae* in liquid fermentation medium

S. HAFIZ, T. O. ODUGBEMI, AND I. GEARY (1979). *Medical Laboratory Sciences*, **36**, 91-94

Using a liquid fermentation medium for the sugar fermentation reactions of *Neisseria gonorrhoeae*, all the 110 strains of gonococci tested produced detectable acid from dextrose only whereas 106 strains gave positive results on standard solid fermentation medium. The medium has the added advantage for clinical laboratories that it does not require incubation in a CO_2 atmosphere. The importance of non-toxic

and growth-supporting fermentation media in the identification of gonococci is discussed.

Authors' summary

Classes of antibodies in acute gonorrhoea

C. A. ISON AND A. A. GLYNN (1969). *Lancet*, **1**, 1165-1168

Using an enzyme-linked immunosorbent assay (Elisa) with outer membrane protein as antigen, the authors examined sera from 381 men and 437 women (191 men and 212 women with gonorrhoea; 106 normal men and 131 normal women; 84 men and 94 women who attended a sexually transmitted diseases clinic but who had no evidence of gonococcal infection). The geometric mean serum level of IgG, IgA, and 'secretory' antibodies in patients with gonorrhoea were two to four times those of the normal controls; the difference was most marked in IgG. A significant increase in IgM antibody was noted in the serum from women, but not men, with gonorrhoea.

Although the mean IgG antibody level in serum from normal men and those attending a clinic was similar, the IgA and secretory antibody levels were significantly higher in the latter group of patients; this difference was not found in female patients.

IgA antibody levels in serum from patients who had previously been infected were significantly higher than in serum from those experiencing their first infection.

A. McMillan

Comparative susceptibility of penicillinase-positive and-negative *Neisseria gonorrhoeae* to 30 antibiotics

W. H. HALL, E. A. SCHIERL, AND J. E. MACCARI (1979). *Antimicrobial Agents and Chemotherapy*, **15**, 562-567

The minimum inhibitory concentrations (MICs) of 30 antibiotics were determined by the agar dilution method for 17 penicillinase-positive and 50 penicillinase-negative strains of *Neisseria gonorrhoeae*. The latter included 42 strains that were penicillin susceptible (pen S) (MIC $< 0.125 \mu g/ml$) and eight strains with intermediate resistance to penicillin (pen I, MIC $0.125-0.5 \mu g/ml$). Two penicillinase-resistant penicillins (methicillin and nafcillin) were inhibitory for penicillin-resistant (pen R) strains. Three new cephalosporins (cefuroxime, cefamandole, cefaclor) and a cephamycin (cefoxitin) were

bacteriostatic (MIC $<0.8 \mu\text{g/ml}$) for 90% of pen S, pen I, and pen R strains. Pen I strains were more resistant than pen R strains to six of 13 cephalosporins. Rifampicin, erythromycin, spectinomycin, chloramphenicol, and the tetracyclines were inhibitory for both pen S and pen R strains. The minimum bactericidal concentrations of cefuroxime, cefamandole, cefaclor, and cefoxitin were measured for 17 pen R strains and eight pen I strains by serial dilution of the antibiotics in trypticase soy broth supplemented with 1% IsoVital X and 1% haemoglobin. All tubes were subcultured after overnight incubation at 37°C . Cefuroxime and cefoxitin were bactericidal at low concentrations (minimum bactericidal concentration $<1.0 \mu\text{g/ml}$) for 16 of 17 pen R strains and six of eight pen I strains.

Authors' summary

Pharmacokinetic determinants of penicillin cure of gonococcal urethritis

H. W. JAFFE, A. L. SCHROETER, G. H. REYNOLDS, A. A. ZAIDI, J. E. MARTIN, AND J. D. THAYER (1979). *Antimicrobial Agents and Chemotherapy*, 15, 587-591

In a study of the pharmacokinetic determinants of penicillin cure of gonococcal urethritis, 45 male volunteers were experimentally infected with strains of *Neisseria gonorrhoeae* having known *in-vitro* penicillin susceptibility. After developing urethritis, subjects received intramuscular penicillin G and had serum samples obtained serially to determine penicillin concentrations. Using a multiple regression technique, the authors studied patient-associated parameters and parameters of the serum penicillin curves to determine the best predictors of treatment results. Cure was best predicted by the time the serum penicillin concentration remained above three to four times the penicillin minimum inhibitory concentration of the infecting strain (probability of correct classification, >0.80). Those cured had serum penicillin concentrations which remained in this range for means of 7-10 h. The findings confirm principles of antimicrobial therapy derived from animal models.

A. McMillan

Antibiotic susceptibility of *Neisseria gonorrhoeae* strains from Europe and Asia

P. PIOT, E. VAN DYCK, J. COLAERT, J.-P. URSI, E. BOSMANS, AND A. MEHEUS (1979). *Antimicrobial Agents and Chemotherapy*, 15, 535-539

In-vitro antimicrobial activity of piperacillin and seven other β -lactam antibiotics against *Neisseria gonorrhoeae* and *Haemophilus influenzae* including β -lactamase-producing strains

C. THORNSBERRY AND C. N. BAKER (1979). *Journal of Antimicrobial Chemotherapy*, 5, 137-142

Pharyngeal *Neisseria gonorrhoeae*: coloniser or pathogen?

J. WALLIN AND M. S. SIEGEL (1979). *British Medical Journal*, 1, 1462-1463

Molecular characterization of a small *Haemophilus influenzae* plasmid specifying β -lactamase and its relationship to R factors from *Neisseria gonorrhoeae*

R. LAUFS, P. M. KAULFERS, G. JAHN, AND V. TESCHNER (1979). *Journal of General Microbiology*, 111, 223-231

In-vitro activity of HR 756, a new cephalosporin against *Neisseria gonorrhoeae*

P. R. MURRAY, J. L. CHRISTMAN, AND G. MEDOFF (1979). *Antimicrobial Agents and Chemotherapy*, 15, 452-454

Penicillinase-producing *Neisseria gonorrhoeae*. New York City 1976-1978

Y. M. FELMAN AND R. SNYDER (1979). *New York State Journal of Medicine*, 79, 697-699

Acridine orange staining of urethral and cervical smears for the diagnosis of gonorrhoea

U. FORSUM AND A. HALLEN (1979). *Acta Dermatovenereologica (Stockholm)*, 59, 281-282

Comparison of three methods for identification of pathogenic *Neisseria* species

P. C. APPELBAUM AND R. B. LAWRENCE (1979). *Journal of Clinical Microbiology*, 9, 598-600

Complement-enhanced immunity to infection with *Neisseria gonorrhoeae* in mice

J. R. ARKO, K. H. WONG, F. J. STEURER, AND W. O. SCHALLA (1979). *Journal of Infectious Diseases*, 139, 569-574

Nuclease enhancement of specific cell agglutination in a serodiagnostic test for *Neisseria gonorrhoeae*

R. J. ARKO, K. H. WONG, AND W. L. PEACOCK (1979). *Journal of Clinical Microbiology*, 9, 517-519

Ngo II, a restriction endonuclease from *Neisseria gonorrhoeae*

D. J. CLANTON, W. S. RIGGSBY, AND R. V. MILLER (1979). *Journal of Bacteriology*, 137, 1299-1307

Type-14 pneumococcal vaccine for prevention of gonorrhoea (letter)

B. B. DIENA, F. E. ASHTON, AND M. B. PERRY (1979). *Lancet*, 1, 1037

Purification by affinity chromatography and properties of a β -lactamase isolated from *Neisseria gonorrhoeae*

L. A. ERIQUEZ AND R. F. D'AMATO (1979). *Antimicrobial Agents and Chemotherapy*, 15, 229-234

Gonorrhoea (Therapy)

A clinical dose-response study of bacampicillin in uncomplicated gonorrhoea

S. BENGTSSON, G. ERIKSON, L.-O. KALLINGS, I. MOBERG, E. SANDSTROM, J. WALLIN, AND G. WALLMARK. *Journal of Antimicrobial Agents and Chemotherapy*, 5, 211-218

In a double-blind study of dose-response, 883 patients with uncomplicated gonorrhoea were given single oral doses of 400, 800, or 1600 mg bacampicillin, with 1 g probenecid. Doses of 800 and 1600 mg were equally effective, giving negative culture results in 98.2% and 99.7% of patients respectively; 400 mg gave negative culture results in 95.2%. The differences between the lowest dose and the two others were statistically significant. In patients with less sensitive strains, the highest dose was more effective. It is concluded that a single dose of bacampicillin 800 mg plus probenecid 1 g was effective in uncomplicated gonorrhoea with fully ampicillin-sensitive strains. In patients with gonococci showing reduced sensitivity to ampicillin the best results (96.3%) were achieved with the double dose of bacampicillin.

Authors' summary

A trial of minocycline given after exposure to prevent gonorrhoea

W. O. HARRISON, R. R. HOOPER, P. J. WIESNER, AND OTHERS (1979). *New England Journal of Medicine*, 300, 1074-1078

In an evaluation of antibiotic prophylaxis against gonorrhoea, 1080 men were given

200 mg of oral minocycline or placebo after sexual intercourse with prostitutes in a Far Eastern port. Later, at sea, gonococcal infection was detected in 57 of 565 men given placebo and 24 of 515 men given minocycline ($p < 0.001$). Minocycline prophylaxis completely prevented infection by gonococci susceptible to 0.75 µg or less of tetracycline/ml, reduced the risk of infection or prolonged the incubation period in men exposed to gonococci susceptible to 1.0-2.0 µg/ml, but did not prevent infection or prolong incubation in men exposed to gonococci resistant to 2.0 µg. Minocycline did not increase the proportion of asymptomatic infections. Minocycline prophylaxis would probably have limited the effectiveness as a public health measure because of the tendency to select resistant gonococci.

A. McMillan

Treatment of gonorrhoea (Editorial)
W. McCORMACK (1979). *Annals of Internal Medicine*, **90**, 845-846

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Candidosis

A study of 666 strains of *Candida albicans*: correlation between serotype and susceptibility to 5-fluorocytosine
P. AUGER, C. DUMAS, AND J. JOLY (1979). *Journal of Infectious Diseases*, **139**, 590-594

Candidal vaginitis. A study of the efficacy of a reduced duration treatment with miconazole nitrate
S. R. MAYHEUR AND W. E. SUFFIELD (1979). *Practitioner*, **222**, 564-567

The effect of 5-fluorocytosine in the blastospores and hyphae of *Candida albicans*
A. POLAK AND W. H. WAIN (1979). *Journal of Medical Microbiology*, **12**, 83-97

Genital herpes

Relation of HVH-2 to carcinoma of the cervix

S. M. TOBIN, E. N. FISH, N. B. E. COOTER, AND F. R. PAPSIN (1979). *Obstetrics and Gynecology*, **53**, 553-558

An animal model demonstrated the development of varying degrees of basal cell hyperplasia, metaplasia, and dysplasia following cervicovaginal herpesvirus hominis type 2 (HVH-2) infection. Although the study spanned a period of 30 months, the equivalent of 60 human years, there was no evidence of anaplasia. These results are considered in relation to the role of HVH-2 as an initiator in the multistep process leading to cervical carcinoma.

Authors' summary

Organ culture model for the study of HVH-2 infections in carcinoma of the cervix

S. M. TOBIN, E. N. FISH, W. D. WILSON, AND F. R. PAPSIN (1979). *Obstetrics and Gynecology*, **53**, 559-564

An animal model demonstrated that after cervicovaginal inoculation infective doses of herpesvirus hominis type 2 induced a form of herpetic encephalitis. Immunofluorescent results indicated that there was both neural and haematogenous spread of the herpes infection. The possible role of a latent viral infection in the genesis of an acute disease of the central nervous system is investigated in this experimental system.

Authors' summary

Genital herpes simplex virus (HSV) isolation during pregnancy

D. A. BAKER AND S. A. PLOTKIN (1979). *Obstetrics and Gynecology*, **53** (Supplement), 95-125

Eight pregnant women from whom herpes virus hominis had been isolated were studied. Viral culture of material from the cervix and vulva was undertaken at intervals. It was shown that, in each case, cultures gave negative results as the pregnancy progressed. Each child was delivered vaginally; none developed lesions of neonatal herpes.

A. McMillan

Ascending infection following cervicovaginal exposure to herpesvirus hominis type 2

E. N. FISH, S. M. TOBIN, W. D. WILSON, AND F. R. PAPSIN (1979). *Obstetrics and Gynecology*, **53**, 429-436

Cesarian section and genital herpesvirus infection (editorial)

M. S. AMSTEY, G. R. G. MONIF, A. J. NAHMIA, AND W. E. JOSEY (1979). *Obstetrics and Gynecology*, **53**, 641-642

Immune control of herpesvirus latency

L. A. BABIAK AND B. T. ROUSE (1979). *Canadian Journal of Microbiology*, **25**, 267-274

Thin-layer immunoassay for determination of antibodies to herpes simplex virus

S. JEANSSON, H. ELWING, AND L. A. NILSSON (1979). *Journal of Clinical Microbiology*, **9**, 317-322

Comparison of indirect haemagglutination and indirect immunofluorescence tests with microneutralisation tests for detection of type-specific herpesvirus hominis antibody

L. D. JOHNSON, D. A. FUCILLO, H. STALDER, M. A. OXMAN, C. E. O. FRASER, AND D. L. MADDEN (1979). *Journal of Clinical Microbiology*, **9**, 384-390

Persistence, reactivation, and cell transformation by human herpesviruses: herpes simplex 1, 2 (HSV-1, HSV-2), cytomegalovirus (CMV), varicella-zoster (VZC), Epstein-Barr virus (EBV)

J. H. JONCAS (1979). *Canadian Journal of Microbiology*, **25**, 254-260

A rapid technique for distinguishing herpes simplex virus type 1 from type 2 by restriction-enzyme technology

D. M. LONSDALE (1979). *Lancet*, **1**, 849-852

Effect of pyran on latency after herpes simplex virus infections

P. S. MORAHAH, P. F. CLINE, M. C. BREINIG, AND B. K. MURRAY (1979). *Antimicrobial Chemotherapy*, **15**, 547-553

Evaluation of indirect haemagglutination and its inhibition in the differentiation between antibodies to herpes simplex with virus types 1 and 2 for seroepidemiologic studies: use of a II/I index threshold of 85 and an assay of type-specific antibodies

S. S. PRAKASH AND P. SETH (1979). *Journal of Infectious Diseases*, **139**, 524-528

Replication of herpesviruses and latency
L. J. ROSENTHAL (1979). *Canadian Journal of Microbiology*, **25**, 239-244

Other sexually transmitted diseases

Prevention of type B hepatitis

J. H. HOOFGUE (1979). *Gastroenterology*, **76**, 1483-1485

Latency and activation of cytomegalovirus in man and mice

S. MONTPLAISIR (1979). *Canadian Journal of Microbiology*, **25**, 261-266

Miscellaneous

Quantitative bacteriology of the vaginal flora in vaginitis

M. E. LEVISON, I. TRESTMAN, R. QUACH, C. SLADOWSKI, AND C. N. FLORO (1979). *American Journal of Obstetrics and Gynecology*, **133**, 139-144

Vaginal flora was studied quantitatively in 29 sexually active women between 16 and 33 years to define the role of *Corynebacterium vaginale* in vaginitis. Seventeen women were asymptomatic and 12 complained of

symptoms of vaginitis. Seven asymptomatic women had scant secretions: four of these seven had *C. vaginale* at $\log_{10}$ 6-9 cfu/ml, none of whom had 'clue' cells; none had *Trichomonas vaginalis* or *Candida albicans*; six had *Lactobacilli* at $\log_{10}$ 7-9.7 cfu/ml; only one had *Bacteroidaceae* at $>\log_{10}$ 5/ml. Ten asymptomatic women had easily collectable secretions; eight of 10 had *C. vaginale* at $\log_{10}$ 6.5-9.6/ml, three of whom had 'clue' cells; four had *T. vaginalis* and none *C. albicans*; nine had *Lactobacilli* at $\log_{10}$ 7-9.3/ml; four had *Bacteroidaceae* at $>\log_{10}$ 5/ml. Twelve had vaginitis: five of 12 had *C. vaginale* at $\log_{10}$ 7.9-11/ml, one of whom had 'clue' cells; nine had either *T. vaginalis* or *C. albicans* or both and three had no pathogen including *C. vaginale*; 10 had *Lactobacilli* at $\log_{10}$ 7-10/ml; six had *Bacteroidaceae* at $>\log_{10}$ 5/ml. Three had 'clue' cells in absence of *C. vaginale*.

Authors' summary

Behcet's syndrome: a family study and the elucidation of a genetic role

L. BERMAN, B. TRAPPLER, AND T. JENKINS (1979). *Annals of Rheumatic Diseases*, **38**, 118-121

Epidemiologic approach to control of sexually transmitted disease

Y. M. FEHMAN AND J. A. NIKITAS (1979). *New York State Journal of Medicine*, **79**, 745-746

Reliability of a single urine culture in establishing diagnosis of asymptomatic bacteruria in adult males

R. GLECKMAN, A. ESPOSITO, M. CROWLEY, AND G. A. NATSIOS (1979). *Journal of Clinical Microbiology*, **9**, 596-597

Vaginitis

J. K. HURD (1979). *Medical Clinics of North America*, **63**, 423-432

Amine content of vaginal fluid from untreated and treated patients with non-specific vaginitis

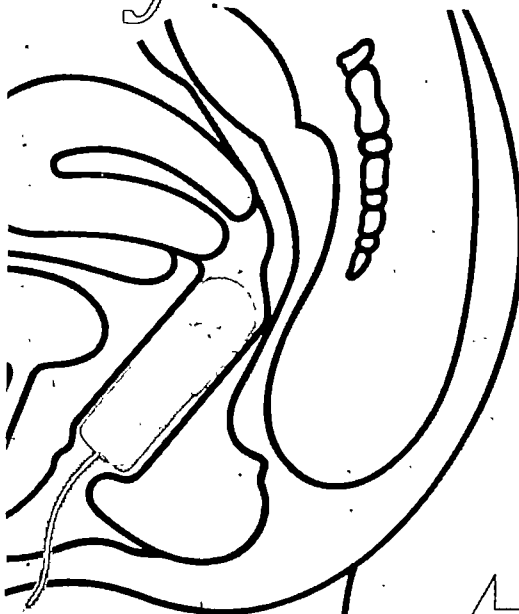
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Venereal infections in children

C. Q. McCLELLAND, M. UYEKI, D. ZEMAITYTE, AND D. E. TINKER (1979). *Journal of the American Medical Association*, **241**, 2141

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28th March, 1980	Chlamydia-Neonatal Infection
	Treatment
25th April, 1980	Teaching of Venereology to Undergraduates
16th-18th May, 1980	Spring Meeting in BOURNEMOUTH

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Contents

Study of STDs in patients attending venereal disease clinics in Khartoum, Sudan
O. M. A. TAHA, M. HAG ALI, E. E. OMER, M. A. AHMED, AND S. A. ABBARO (Sudan) *page 313*

Use of CB hamsters in the study of *Treponema pertenue*
R. F. SCHELL, J. L. LE FROCK, J. P. BABU, AND J. K. CHAN (USA) *page 316*

Cultivation of *Treponema pallidum* in subcutaneous chambers implanted in golden hamsters
G. D. MORRISON, S. I. EGGLESTONE, AND J. L. NORTHWOOD (Gt Britain) *page 320*

Sensitivity of *Neisseria gonorrhoeae* to antibiotics in London (1976-78)
A. D. SETH, B. KOLATOR, AND A. E. WILKINSON (Gt Britain) *page 325*

Failure of iron to promote attachment of gonococci to human spermatozoa under physiological conditions
A. P. JOHNSON AND MARY F. OSBORN (Gt Britain) *page 329*

Frequency of simultaneous carriage of *Neisseria gonorrhoeae* and *Neisseria meningitidis*
K. ØDEGAARD AND T. W. GEDDE-DAHL (Norway) *page 334*

Meningococcal colonisation misdiagnosed as gonococcal pharyngeal infection
R. C. NOBLE AND R. M. COOPER (USA) *page 336*

Double-blind trial of amoxycillin and ampicillin plus probenecid in the treatment of gonorrhoea in men
A. R. BRATHWAITE (Jamaica) *page 340*

Single-dose minocycline in the treatment of gonococcal urethritis
P. M. WATERWORTH, J. D. ORIEL, G. L. RIDGWAY, AND S. SUBRAMANIAN (Gt Britain) *page 343*

Vaginal oxytetracycline concentrations
R. N. THIN, Z. H. AL RAWI, P. D. SIMMONS, J. TREHARNE, AND S. TABAQCHALI (Gt Britain) *page 348*

Metronidazole-resistant *Trichomonas vaginalis*
A. FORSGREN AND L. FORSSMAN (Sweden) *page 351*

Double-blind comparison of a single dose and a five-day course of metronidazole in the treatment of trichomoniasis
R. N. THIN, M. A. E. SYMONDS, R. BOOKER, S. COOK, AND F. LANGLET (Gt Britain) *page 354*

Epidemiological investigation of patients with vulvovaginal candidosis
D. W. WARNOCK, D. C. E. SPELLER, J. D. MILNE, A. L. HILTON, AND P. I. KERSHAW (Gt Britain) *page 357*

Effect of simultaneous oral and vaginal treatment on the rate of cure and relapse in vaginal candidosis
J. D. MILNE AND D. W. WARNOCK (Gt Britain) *page 362*

Sexual transmission of hepatitis B surface antigen
N. INABA, R. OHKAWA, A. MATSUURA, J. KUDOH, AND H. TAKAMIZAWA (Japan) *page 366*

Staphylococcus saprophyticus in the aetiology of nongonococcal urethritis
B. HOVELIUS, I. THELIN AND P.-A. MÅRDH (Sweden) *page 369*

Epidemic of amoebiasis and giardiasis in a biased population
B. H. KEAN, D. C. WILLIAM, AND S. K. LUMINAIS (USA) *page 375*

Correspondence *page 379*

Book reviews *page 381*

Abstracts *page 382*

ASTM CODEN: BJVDK 55 (5) 313-386 (1979)

ISSN 0007-134X

PUBLISHED BY BRITISH MEDICAL ASSOCIATION, TAVISTOCK SQUARE, LONDON WC1H 9JR, AND
PRINTED IN ENGLAND BY DAWSON & GOODALL LTD., THE MENDIP PRESS, BATH

29.4.80
December 1979 Vol. 55 No. 6

British Journal of Venereal Diseases

BRITISH MEDICAL ASSOCIATION TAVISTOCK SQUARE LONDON WC1

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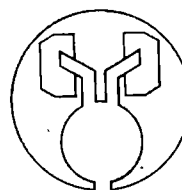
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References

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Reference: 1 Balmer, J A (1976) Am J Obstet Gynaec 126, 4, 436-441

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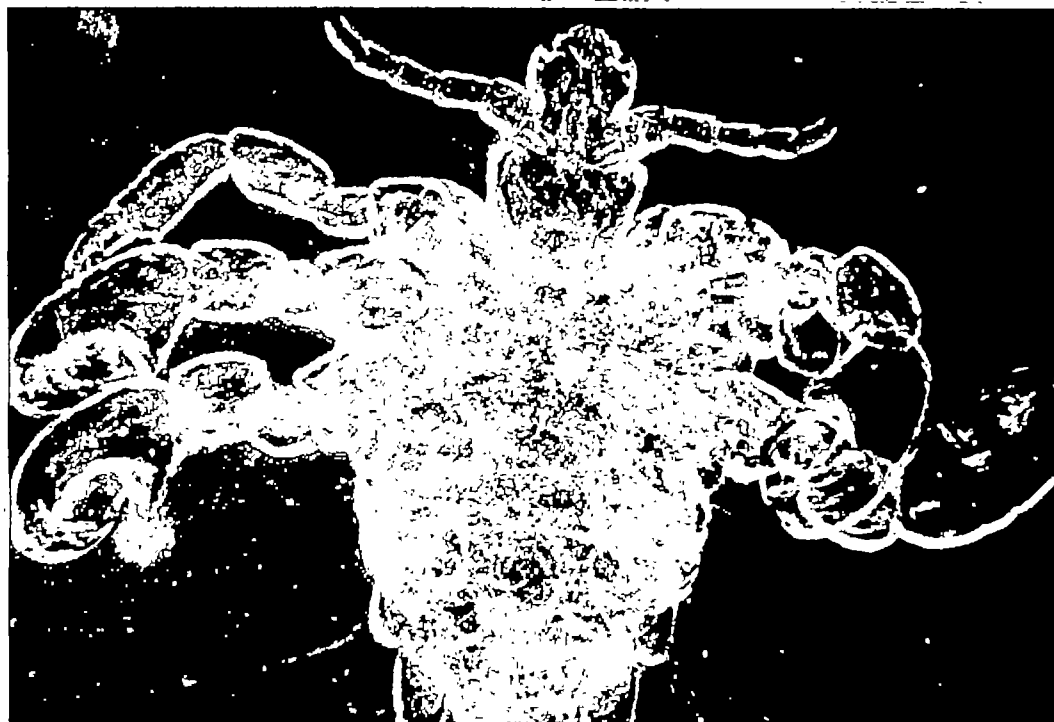
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2 Brit. J. Vener. Dis. (1975), 51, 206-209

3 Brit. J. Vener. Dis. (1976), 52, 43-45

4 Brit. J. Vener. Dis. (1978), 54, 107-111

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Optimum concentration of dissolved oxygen for the survival of virulent *Treponema pallidum* under conditions of low oxidation-reduction potential

S. GRAVES AND T. BILLINGTON

From the Department of Microbiology, Monash University Medical School, Alfred Hospital, Prahran, Australia

SUMMARY A maintenance medium with a low oxidation-reduction (redox) potential, when gently bubbled with 5% oxygen in nitrogen or with air for various periods of time, gave a range of dissolved oxygen concentrations between 1.6 and 5.8 $\mu\text{g/l}$. Virulent *Treponema pallidum* (Nichols strain) inoculated into these media were assayed 24 and 48 hours later for motility and virulence and were compared with samples taken at zero time. Virulent *T. pallidum* survived best in the presence of 2.4 $\mu\text{g/l}$ dissolved oxygen over a 48-hour period, which corresponded to a gaseous mixture of 3% oxygen in nitrogen. Higher concentrations of oxygen did not give significantly different results from anaerobic conditions over this period. Thus, until it can be grown *in vitro*, *T. pallidum* would appear to be a microaerophilic bacterium.

Introduction

During this century attempts to grow virulent *Treponema pallidum* (the causative agent of syphilis) *in vitro* have been unsuccessful despite claims to the contrary (Willcox and Guthe, 1966). Growth of avirulent *T. pallidum* from a virulent inoculum has recently been reported (Sandok *et al.*, 1976, 1978). Since the availability of large numbers of *T. pallidum* which are relatively free from host-cell contaminants is probably a prerequisite for the development of a vaccine against syphilis, attempts to grow this bacterium *in vitro* are important steps in reducing the incidence of this disease.

For many years *T. pallidum* has been considered a strict anaerobe with a low oxidation-reduction (redox) optimum (Metzger and Smogor, 1966). Only in reduced media could the organism maintain its viability, as measured by rabbit virulence. Recently this opinion has been challenged. Freshly isolated *T. pallidum* utilises oxygen at a rate equivalent to that of aerobic organisms (Cox and Barber, 1974), and the presence of a cytochrome system (Lysko and

Cox, 1977), suggests that this organism may respire aerobically. Glucose oxidation, pyruvate degradation, and protein synthesis were stimulated under aerobic conditions (Baseman *et al.*, 1976). Norris *et al.* (1978), using a reduced culture medium, found that virulent *T. pallidum* survived better under 3% oxygen than under either aerobic or anaerobic conditions, indicating that the bacterium is microaerophilic.

The importance of oxygen for the survival and metabolism of *T. pallidum* has also been shown in tissue culture systems where the addition of superoxide dismutase to infected cell monolayers prolonged the virulence of *T. pallidum* (Fitzgerald *et al.*, 1975). With certain cell lines and reduced culture medium an atmosphere of 3% oxygen maintained *T. pallidum* without loss of virulence for six days (Fitzgerald *et al.*, 1977) and 2.5% oxygen enabled a surface acid mucopolysaccharide to be synthesised as a result of the cell-treponeme interaction (Fitzgerald *et al.*, 1978). Fieldsteel *et al.* (1977) established an oxygen gradient in tissue culture and found that *T. pallidum* survived best in a region neither fully aerobic nor anaerobic, indicating the importance of microaerobiosis for the survival of *T. pallidum*. Kiraly and Horvath (1976) used low oxygen conditions (7 mm Hg partial pressure) and found that *T. pallidum* survived well, but in their work

Address for reprints: Dr S Graves, Department of Microbiology, Monash University Medical School, Alfred Hospital, Prahran, Victoria 3181, Australia

Received for publication 27 April 1979

survival was poorer with, rather than without, a tissue culture system.

In our study, an optimum dissolved oxygen concentration of $2.4 \mu\text{g/l}$ (corresponding to a gaseous mixture of 3% O_2 in N_2) was required for *T. pallidum* to retain its virulence.

Materials and methods

PROPAGATION OF *T. PALLIDUM*

The bacterium was grown in the testes of serologically negative rabbits by inoculating approximately 5×10^7 viable *T. pallidum* per testis and harvesting it 11 days later, at which time orchitis has developed. Rabbits (of various breeds) were housed at 18°C to permit satisfactory growth of the *T. pallidum*. After the rabbit had been killed with intravenous sodium pentobarbitone, the testes were removed aseptically and minced in successive 5-ml samples of medium (see below) under aerobic conditions. This eluted the *T. pallidum* from the tissue, yielding about 10^7 organisms/ml. The suspension was used without purification and 0.5 ml was inoculated into 10-ml tubes of medium to give a final concentration of *T. pallidum* of about $5 \times 10^5/\text{ml}$. This concentration varied from experiment to experiment depending on the concentration of treponemes in the rabbit testes. The 1/20 dilution of the inoculum into experimental medium meant that the treponemes were only exposed to air, albeit in a reduced medium, for about 30 minutes.

T. PALLIDUM IN VITRO

After inoculation of the medium with *T. pallidum* under a stream of sterile, oxygen-free nitrogen the tubes were tightly sealed with butyl rubber stoppers (Bellco Glass Inc., Vineland, New Jersey), placed in anaerobic jars to prevent extraneous oxygen entering the medium, and incubated at 35°C .

PREPARATION OF MEDIUM

The medium of Graves *et al.* (1975) was modified and made in two parts (A and B), which were combined immediately before inoculation with *T. pallidum*. The compositions of medium A and B are shown in Table 1. Medium A (without buffered bicarbonate solution or reducing agents) was prereduced by autoclaving at 121°C for eight minutes with slow exhaust. Immediately after removal from the autoclave the medium was continuously flushed with oxygen-free nitrogen until cool; the latter was obtained by passing nitrogen through a hot copper column before use. When the medium was cool, buffered bicarbonate solution and reducing agents (as powders) were added by gently stirring. The pH

Table 1 Composition of *T. pallidum* maintenance medium

Component	Amount/l (Final total medium)
Medium A	
Balanced salt solution concentrate ($10 \times$)*	60 ml
$(\text{NH}_4)_2\text{SO}_4$ (50 g/l)	2 ml
Glucose	1.60 g
Fructose	1.60 g
H_2O	400 ml
Buffered bicarbonate solution†	10 ml
Glutathione (reduced)	2.38 g
Cysteine	1.58 g
Sodium thioglycolate	1.00 g
Resazurin solution (0.25 g/l)	4 ml
Medium B	
Glutamine (29.2 g/l)	10 ml
Amino acid concentrate A‡	10 ml
Amino acid concentrate B§	10 ml
Amino acid concentrate C	10 ml
Vitamin concentrate¶	10 ml
Solution D**	10 ml
Foetal calf serum (heat inactivated)	250 ml
H_2O	220 ml

*NaCl (40 g/l), KCl (4 g/l), CaCl_2 (1.4 g/l), Na_2HPO_4 (0.6 g/l), KH_2PO_4 (0.6 g/l), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (2 g/l)

† NaHCO_3 (50 g/l), K_2HPO_4 (40 g/l) and KH_2PO_4 (8 g/l)

‡Composition per 10 ml: arginine HCl, 105 mg; histidine HCl H_2O , 31 mg; isoleucine, 52 mg; leucine, 52 mg; lysine HCl 58 mg; methionine, 15 mg; phenylalanine, 32 mg; threonine, 48 mg; tryptophan, 10 mg; and valine, 46 mg

§Composition per 10 ml: cystine, 24 mg and tyrosine, 36 mg

||Composition per 10 ml: alanine, 8.9 mg; asparagine H_2O , 15 mg; aspartic acid, 13.3 mg; glutamic acid, 14.7 mg; proline, 11.5 mg; serine, 10.5 mg; and glycine, 7.5 mg

¶Composition per 10 ml: choline chloride, 1 mg; folic acid, 1 mg; 1-inositol, 2 mg; nicotinamide, 1 mg; calcium pantothenate, 1 mg; pyridoxal HCl, 1 mg; riboflavin, 0.1 mg; and thiamine HCl, 1 mg
**Composition per 10 ml: sodium pyruvate, 250 mg; adenine, 5 mg; coenzyme A, 50 µg; isobutyric acid, 10 mg; α-lipoic acid, 5 mg; p-amino benzoic acid, 5 mg; biotin, 50 µg; nicotinic acid, 500 µg; pyridoxine HCl, 2.5 mg; pyridoxamine HCl, 5 mg; putrescine 2HCl, 10 mg; and cyanocobalamin, 50 µg

of the solution was adjusted to 8.6 with 10 mol/l NaOH. The medium was maintained under a stream of nitrogen until it was added to nitrogen-flushed, anaerobic tubes (Bellco Glass Inc.) using a pipette preflushed with nitrogen (4.7 ml/tube). The tubes were tightly sealed with butyl rubber stoppers and autoclaved in a press at 121°C for 15 minutes using fast exhaust. Medium A was never prepared earlier than one day before use. After medium A had been cooled, medium B was added to the tubes of medium A under sterile, oxygen-free nitrogen. To make 10 ml of complete medium, 5.3 ml of medium B was added to 4.7 ml of medium A. All components of medium B were previously sterilised by filtration. The final pH was 7.2-7.4.

OXYGEN BUBBLING INTO MEDIUM

Cylinders of 5% oxygen in nitrogen (Commonwealth Industrial Gases, Melbourne, Australia) or of air were used to add oxygen to the medium. When medium A had been prepared, and immediately

before it was added to the nitrogen-flushed anaerobic tubes, it was bubbled with the appropriate gas mixture for the predetermined time. For example, 50% $O_2 \times 15$ minutes means 15 minutes' bubbling with 50% O_2 for every 10 ml of medium being prepared, that is, 150 minutes for 100 ml final medium. The bubbling rate was approximately 2 bubbles per second from a 21-mm Pasteur pipette (orifice diameter approximately 1 mm). After bubbling, medium A was dispensed in 4.7-ml volumes into nitrogen-flushed anaerobic tubes and sterilised by autoclaving in a press; medium B was then added as before.

MEASUREMENT OF OXYGEN IN MEDIUM

An oxygen meter (Model LR-30, Titron Australia, Melbourne), fitted with a Clark-type oxygen electrode modified to allow measurement in static solutions, was used. The meter gave readings in 'part per million' ($\mu g/l$) dissolved oxygen in water. The electrode was calibrated at 22°C with air (being the same as air-saturated water at 22°C), 5% O_2 , 3% O_2 , and 1% O_2 in nitrogen and 100% nitrogen (oxygen-free). The exact oxygen concentration in the gas mixtures was determined by the suppliers (Commonwealth Industrial Gases, Melbourne) and was accurate to 0.1%. The relationship between oxygen-meter readings ($\mu g/l$) and O_2 concentration in the gas was linear (Figure); this was the standard

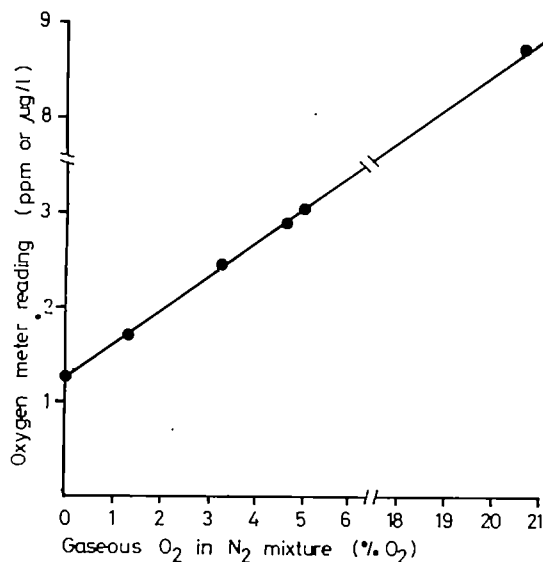


Figure Relationships between oxygen meter reading and concentration of oxygen in the gaseous O_2-N_2 mixture. This standard curve was used to determine the oxygen concentrations in culture media as equivalent gaseous oxygen concentrations.

curve from which all subsequent O_2 concentrations in media were determined. We appreciated that the calibration curve, based on dissolved oxygen concentration in water, was not strictly applicable to the culture medium. Chemical determinations of dissolved oxygen concentrations in the culture media were not done. However the method used enabled the most appropriate gaseous O_2-N_2 mixture for optimum retention of *T. pallidum* virulence in the culture medium to be determined.

Dissolved oxygen measurements on triplicate tubes were performed at daily intervals. Fresh tubes were used for readings at each time interval because insertion of the oxygen electrode biologically contaminated the medium. The electrode was inserted into the bottom of the 10-ml column of medium and stable readings were obtained within one to two minutes. Exposing the medium to air for this short time did not affect the oxygen reading, as confirmed by flushing control tubes with nitrogen while taking oxygen readings.

MEASUREMENT OF REDOX POTENTIAL IN THE MEDIUM

A platinum calomel combination redox electrode (Model 96-78, Orion, Cambridge, Massachusetts) was connected to an Orion model 701A 'digital ionalyser' and inserted directly into the medium for five minutes. Measurements were made at approximately 22°C and pH 7.3. The redox potential was read directly from the millivolt scale of the meter. The efficacy of the electrode was confirmed with a standard redox system of potassium ferricyanide and potassium ferrocyanide as recommended by the manufacturer. The readings obtained, being based on a calomel reference electrode, are designated Ecal; to convert them to Eh values, approximately 241 mV must be added to the reading (Jacob, 1970)—that is, -276 mV (Ecal) is equivalent to -35 mV (Eh).

DETERMINATION OF *T. PALLIDUM* VIRULENCE

Samples (0.6 ml) of culture medium were taken immediately after inoculation with *T. pallidum* and at 24, and 48 hours thereafter. Each sample was mixed with 0.6 ml of sterile deoxygenated 20% glycerol in saline and stored at -70°C until all samples were available. Samples were thawed immediately before inoculation into the rabbit. The backs of male rabbits were shaved and marked into a 10-cm $\times$ 10-cm grid, which was subdivided into 16 equal areas. Into each site 0.1 ml of culture was injected intradermally. For each time sample, quadruplicate samples were injected into each rabbit (4 $\times$ 0 h; 4 $\times$ 24 h; 4 $\times$ 48 h). Rabbits were kept shaved and cool (18°C) and examined daily for

development of lesions as determined by the detection of induration at the site of inoculation, which subsequently developed into a typical syphilitic lesion. Some of these lesions were abraded and shown to contain treponemes. The time the lesions took to develop for the 24-hour and 48-hour samples was compared with that for the zero time samples. Testing all samples from one experiment on the same rabbit overcame the problem of variability between rabbits in their susceptibility to *T. pallidum*.

DETERMINATION OF *T. PALLIDUM* MOTILITY

Darkfield microscopy was used to examine 100 *T. pallidum* chosen at random in a drop of culture medium on a microscope slide. Each bacterium was scored as motile or non-motile and the percentage motility of the culture determined. Only actively motile *T. pallidum* were considered motile—that is, those with snapping, spinning, or flexing motions. For each experiment motility determinations were carried out in triplicate from three identical tubes of media.

Statistical comparisons were made by Student's *t* test.

Results

The anaerobic medium (Table 1) used for the maintenance of *T. pallidum* could be made partially aerobic by bubbling 5% oxygen in nitrogen or air into it for various periods of time. Maximum oxygenation obtained in this series of experiments was 5.8 µg/l, equivalent to an atmosphere of 12.6% oxygen (Table 2) with a series of lower values down to 1.6 µg/l (equivalent to an atmosphere of 0.7% oxygen), the anaerobic medium.

Redox potentials of the anaerobic medium were not affected by bubbling the medium with 5% oxygen in nitrogen (data not shown), but bubbling with air did cause the redox potential to become more positive (Table 3). Bubbling the medium with air for 60 minutes produced the highest redox

Table 2 Measurement of dissolved oxygen concentration in medium* following bubbling with 5% oxygen in nitrogen or air for specified periods

Method of oxygen addition	Dissolved oxygen concentration in µg/l (% O ₂)		
	Day 0	Day 1	Day 2†
No oxygenation	1.6 (0.7)	1.6 (0.7)	1.6 (0.7)
5% O ₂ in N ₂ × 5 min	1.8 (1.3)	1.8 (1.3)	1.7 (1.0)
5% O ₂ in N ₂ × 15 min	2.4 (3.0)	2.2 (2.4)	2.1 (2.1)
Air × 30 min	2.95 (4.7)	2.6 (3.6)	2.4 (3.0)
Air × 40 min	3.8 (7.3)	3.6 (6.5)	3.4 (5.9)
Air × 50 min	5.0 (10.4)	3.6 (6.5)	2.0 (1.9)
Air × 60 min	5.8 (12.6)	4.2 (8.2)	2.1 (2.1)

*Mean of five independent samples. Measurements made immediately after inoculation of medium with *T. pallidum* (day 0) and on days 1 and 2.

†The fall in oxygen concentration over the two-day period is presumably due primarily to removal of the added free oxygen by reducing agents in the medium, as very little would be due to treponemal metabolism.

Table 3 Oxidation reduction (redox) potential (mV) of medium after bubbling with air for specified periods

Method of oxygen addition	Redox potential (mV)*		
	Day 0	Day 1	Day 2
No oxygenation	-276	-272	-254
Air × 30 min	-271	-271	-265
Air × 40 min	-263	-263	-261
Air × 50 min	-259	-258	-227
Air × 60 min	-243	-240	-229

*Mean of four independent samples. Measurements made immediately after inoculation of medium with *T. pallidum* (day 0) and on days 1 and 2.

potentials, but even so the medium was still 'reduced' relative to a calomel reference electrode.

The oxygenation of the medium did not have a marked effect on the reduction in percentage motility of the *T. pallidum* culture (Table 4); by day 2 the more oxygenated cultures were slightly less motile than the anaerobic controls.

Using the intradermal inoculation test on the shaved back of the rabbit, a trend was observed when the cultures were tested for the presence of virulent *T. pallidum*. In anaerobic medium the latent period of infection was shortened by only 0.2 day over the first 24 hours. The only oxygenated media to

Table 4 Determination of motility of *Treponema pallidum* cultures (%) maintained in medium bubbled with 5% oxygen in nitrogen or air for specified periods

Method of oxygen addition	Time of motility estimation*			% decrease (day 0 to day 1)	% decrease (day 0 to day 2)
	Day 0	Day 1	Day 2		
No oxygenation	100	94	80	6	20
5% O ₂ × 5 min	100	96	91	4	9
5% O ₂ × 15 min	100	91	77	9	23
Air × 30 min	100	88	67	12	33
Air × 40 min	100	91	73	9	27
Air × 60 min	100	85	62	15	38

*Mean of three samples from each of three identical tubes of media.

alter significantly the difference in the latent period was after being bubbled with 5% O₂ for 15 minutes (Table 5). After bubbling with 5% O₂ for 15 minutes (that is, 2.4 µg/l dissolved O₂ concentration equivalent to a 3% oxygen atmosphere; Table 2), the difference in latent period was 1.4 days (Table 5).

Table 5 Latent period of syphilitic lesions after inoculation of *T. pallidum* into the shaved backs of rabbits*

Method of oxygen addition	Latent period of lesions (days)†		Difference between day 0 and day 1 latent periods‡ (days)
	Day 0	Day 1	
No oxygenation	10.7	10.5	-0.2
5% O ₂ in N ₂ × 5 min	9.6	9.1	-0.5
5% O ₂ in N ₂ × 15 min	10.4	9.0	-1.4§
Air × 30 min	9.1	7.9	-1.2
Air × 40 min	9.1	8.75	-0.35
Air × 50 min	12.8	11.9	-0.9
Air × 60 min	12.8	12.4	-0.4

*Samples for rabbit inoculation were taken from medium either immediately after addition of *T. pallidum* (day 0) or 24 hours later (day 1) after incubation at 34°C

†Latent period defined as the time between rabbit inoculation and appearance of syphilitic lesion as indicated by induration in the skin. The differences in latent periods of samples taken on day 0 is due to differences in starting concentration of *T. pallidum* from different harvests and to slight differences in susceptibility of rabbits used in different experiments. In each case the data presented are the mean of 16 lesions from four rabbits. Degree of oxygenation of medium would have no effect on latent period of day 0 samples

‡- means that day 1 samples had shorter latent periods than day 0 samples

§When compared by Student's *t* test, the difference was significant $P < 0.05$

When the latent periods at day 0 and 1 were compared by Student's *t* test, the value of 1.4 days was significant at $P < 0.05$. In five repeated experiments using 5% O₂ in N₂ for 15 minutes, the difference in latent period from day 0 to day 1 was always greater than for anaerobic medium, indicating better survival in the slightly oxygenated medium compared with the anaerobic medium.

Over a 48-hour incubation period *in vitro*, the latent period of infection always lengthened (Table 6), indicating a loss of virulent *T. pallidum* during this time. However the lengthening of the latent period with 5% O₂ in N₂ for 15 minutes was only 4.8 days compared with 6.6 days in anaerobic medium. Differences in latent period for media with other levels of oxygenation were not significantly different to the anaerobic control.

We conclude that bubbling the anaerobic medium with 5% oxygen in nitrogen for 15 minutes (per 10-ml medium volume), resulting in a dissolved oxygen concentration of 2.4 µg/l (equivalent to a 3% O₂ in N₂ atmosphere), significantly enhanced the survival of virulent *T. pallidum* over a 48-hour period compared with anaerobic controls. Media with

Table 6 Latent period of syphilitic lesions after inoculation of *T. pallidum* into the shaved backs of rabbits

Method of oxygen addition	Time at which samples taken for rabbit inoculation*†		Difference between day 0 and day 2 latent periods‡ (days)
	Day 0	Day 2	
No oxygenation	10.1	16.7	+6.6
5% O ₂ in N ₂ × 5 min	9.6	15.9	+6.3
5% O ₂ in N ₂ × 15 min	10.4	15.2	+4.8
Air × 30 min	9.0	16.2	+7.2
Air × 40 min	9.0	16.3	+7.3
Air × 60 min	14.5	21.3	+6.8

*Samples for rabbit inoculation were taken from medium either immediately after addition of *T. pallidum* (day 0) or 48 hours later (day 2) after incubation at 34°C

†See Table 5

‡+ means that day 2 samples had longer latent periods than day 0 samples. In each experiment the day 0 and day 2 samples were inoculated into the same rabbit so as to overcome any variability between rabbits

higher concentrations of dissolved oxygen (up to 5.8 µg/l, equivalent to a 12.6% O₂ in N₂ atmosphere) behaved no differently from anaerobic controls regarding retention of *T. pallidum* virulence during this time.

Discussion

From many earlier observations (Willcox and Guthe, 1966) we expected that a dissolved oxygen concentration would be detected above which oxygen was toxic to *T. pallidum*. This is because *T. pallidum* has always retained its virulence best *in vitro* under anaerobic conditions, implying that an atmospheric concentration of oxygen (21%) was toxic to the bacterium. In this study, although we found an atmospheric equivalent of 3% O₂ to be the optimum concentration for the survival of virulent *T. pallidum* (Table 5), higher concentrations (up to 12.6%; Table 2) were no more detrimental to survival than anaerobic conditions. This suggests that *T. pallidum* does have inherent mechanisms for dealing with the toxic end-products of oxygen reduction (Fridovich, 1975, 1978; Haugaard, 1968), a necessary requirement for aerobic metabolism.

Oxygen at atmospheric concentrations (21%) may, however, overwhelm these mechanisms. Since *T. pallidum* is an obligate human parasite it may have partially lost these mechanisms as a result of degenerate evolution associated with specialisation for a parasitic existence. The guanine plus cytosine (G+C) content of *T. pallidum* indicates that it may be closely related to *Spirochaeta zuelzeri* and *Spirochaeta litoralis* (Miao and Fieldsteel, 1978), free-living anaerobic spirochetes. *Spirochaeta aurantia*, which is a facultative anaerobe, may be a close (but non-parasitic) relative of *T. pallidum*.

Under aerobic conditions it incompletely oxidises carbohydrates mainly to acetate and carbon dioxide. It does not possess a tricarboxylic acid (TCA) (Krebs) cycle but has developed mechanisms of oxidative phosphorylation and a rudimentary electron transport chain involving one or two cytochromes (Canale-Parola, 1977).

By comparison, *T. pallidum* degrades glucose and pyruvate to CO₂ and acetate (Nichols and Baseman, 1975) under both aerobic and anaerobic conditions, with better conversion under aerobic conditions (Baseman *et al.*, 1976). It has a functional electron transport system probably driven by the oxidation of reduced nicotinamide-adenine dinucleotide (NADH) and reduced nicotinamide-adenine dinucleotide-phosphate (NADPH) (Lysko and Cox, 1977) and coupled to oxidative phosphorylation with the production of adenosine triphosphate (ATP) (Lysko and Cox, 1978). The production of ATP in cell-free extracts was stimulated by air. The presence of catalase was also inferred by the release of O₂, presumably from H₂O₂ (Lysko and Cox, 1978). Like *S. aurantia*, *T. pallidum* lacks a TCA cycle, thereby probably explaining its slow growth rate (Schiller and Cox, 1977). The most likely origin of *T. pallidum* therefore is from an ancestral spirochete, something like *Spirochaeta aurantia*, capable of growing in and utilising oxygen to a limited extent. Consequently, it is not surprising that *T. pallidum* should have a requirement for an optimum oxygen concentration.

Norris *et al.* (1978), comparing the effect of anaerobic, aerobic, and 3% oxygen conditions in the presence of reducing agents on the survival of virulent *T. pallidum*, found 3% oxygen to be superior.

We have come to the same conclusion after comparing anaerobic conditions and a range of dissolved oxygen concentrations in reduced culture medium. It is probably no coincidence that our determined oxygen optimum of 3% is within the range of normal rabbit tissue (2.6-4% oxygen; Campbell, 1931)—1.5% in the testis (Cross and Silver, 1962)—and human subcutaneous tissue (2.0-3.6% oxygen; SeEVERS, 1936).

Using cell-free extracts, glucose and pyruvate were degraded under aerobic conditions (Nichols and Baseman, 1975). Baseman *et al.* (1976) found optimum substrate degradation and amino acid utilisation with 10-20% O₂. The synthesis of high molecular weight proteins and incorporation of uridine into RNA was detected under normal atmospheric conditions (Baseman and Hayes, 1977; Nichols and Baseman, 1978), incorporation being poorer with lower oxygen concentrations. However there is no evidence that atmospheric oxygen concentration is compatible with the maintenance of

T. pallidum viability as measured by the retention of virulence.

Under anaerobic, reduced conditions amino acids have been incorporated into protein (Baseman and Hayes, 1974) and serine and uracil taken up by *T. pallidum* (Sandok and Jenkin, 1978).

It appears likely that when *T. pallidum* is successfully grown *in vitro*, it will grow in quite a wide range of oxygen concentrations, but from our present state of knowledge studies on the *in-vitro* growth of this bacterium should be conducted under microaerophilic conditions.

It is interesting that a human malaria protozoon (*Plasmodium falciparum*), another human parasite which defied cultivation *in vitro* for many years, has recently been grown in a culture system with 1% or 5% oxygen (Trager and Jensen, 1976).

We wish to thank Mr Ian McLean for his excellent technical assistance and Dr J. Watterson of Monash University, Department of Mathematics, for valuable help with the statistical analysis.

This work was supported by grants from the National Health and Medical Research Council, the Utah Foundation, the Danks Trust, the estate of the late George Adams, the Ian Potter Foundation, and Monash University, from whom funds are gratefully acknowledged.

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Susceptibility of rabbits to *Treponema pallidum* after infection with *Mycobacterium bovis*

S. GRAVES

From the Department of Microbiology, Monash University Medical School, Alfred Hospital, Prahran, Australia

SUMMARY Rabbits stimulated with *Mycobacterium bovis* (strain BCG) one month before challenge with *Treponema pallidum* (Nichols) did not show any modification in their development of syphilitic lesions. A second infection with BCG given at the same time and at the same intradermal site as the *T. pallidum* challenge also failed to protect the rabbits against syphilis. Thus the non-specific activation of cell-mediated immunity by BCG does not appear to protect rabbits against *T. pallidum* infection even when both activation and challenge take place in the dermis. The role of the macrophage in syphilis remains obscure.

Introduction

Human syphilis and the animal model disease (*Treponema pallidum* infection in rabbits) results in both a humoral and a cell-mediated immune response by the host (Kiraly, 1976; Musher *et al.*, 1976), but the relative contribution of each to overall immunity has yet to be determined. Antibody alone is not fully protective, since in passive transfer experiments rabbits ultimately succumb to infection (Perine *et al.*, 1973; Sepetjian *et al.*, 1973; Turner *et al.*, 1973; Graves and Johnson, 1975; Bishop and Miller, 1976; Weiser *et al.*, 1976). This suggests that cell-mediated immune responses also play a part in the establishment of the immune state. There is evidence both for and against this (Levene *et al.*, 1971; Metzger and Smogor, 1975; Schell and Musher, 1975; Schell *et al.*, 1975a, b; Wicker and Wicker, 1975; Baughn *et al.*, 1977b; Metzger *et al.*, 1977; Wicker and Wicker, 1977).

Although activation of host macrophages is one of the main effector mechanisms of cell-mediated immunity, resulting in enhanced intra-macrophage killing of phagocytosed micro-organisms, it has been shown that the non-specific activation of rabbit macrophages by living *Mycobacterium bovis* (strain BCG) or *Propionibacterium acnes* did not render rabbits more resistant to a *T. pallidum* challenge

(Graves and Johnson, 1975; Schell *et al.*, 1975b; Baughn *et al.*, 1977a).

The experiment reported here was devised to stimulate host immunity with BCG at the same site as the *T. pallidum* infection, the dermis. It was thought that the activation of the rabbit macrophages in the dermis by injection of *Mycobacterium tuberculosis* (BCG strain) at that site would be more protective than the previously used technique of intravenous injection of BCG (Graves and Johnson, 1975), resulting mainly in activated macrophages in liver and spleen. This is because the *T. pallidum* challenge is normally given intradermally into the shaved back of the rabbit. Lukehart and Miller (1978) have previously commented on the need to activate macrophages at the actual site of *T. pallidum* challenge before drawing conclusions about their function in this disease. They have demonstrated macrophage uptake of *T. pallidum* *in vitro*.

As reported in this paper, rabbits stimulated with BCG were still infected with *T. pallidum* and no protection was evident, suggesting that activated macrophages do not play a significant part in resistance to reinfection in rabbit syphilis. Baughn *et al.* (1977a) came to a similar conclusion after intradermally injected *Propionibacterium acnes* failed to protect rabbits against an intradermal challenge with *T. pallidum*.

Materials and methods

All rabbit groups (A, B, C, D, and E) contained three rabbits (Table).

Address for reprints: Dr S. Graves, Department of Microbiology, Monash University Medical School, Alfred Hospital, Prahran, Victoria 3181, Australia

Received for publication 21 February 1979

Table Inoculation of five groups of rabbits with *Mycobacterium bovis* (BCG) and *Treponema pallidum*

Experimental groups	Inoculation time			
	Zero	One month*	Six months	
	BCG (2 mg i.v.)	BCG†	<i>T. pallidum</i> ‡	<i>T. pallidum</i> §
A	Yes	Yes	Yes	Yes
B	Yes	No	Yes	Yes
C	No	Yes	Yes	Yes
D	No	No	Yes	Yes
E	No	No	No	Yes

*In groups A and C the BCG and *T. pallidum* challenge was given simultaneously in the same syringe after being mixed within five minutes of inoculation.

†Eight sites were inoculated; 250 µg/site, total 2 mg.

‡Eight sites were inoculated; two each with 10⁴, 10³, 10², and 10 intradermally.

§Eight sites were inoculated; two each with 10⁶, 10⁵, 10⁴, and 10³ intradermally.

INFECTION WITH BCG

At zero time

Rabbits in groups A and B (Table) were pretreated with 2 mg BCG (dry weight) given intravenously in saline one month before the first challenge with *T. pallidum*. BCG was obtained from the Commonwealth Serum Laboratories, Melbourne, Australia.

At one month

Rabbits in groups A and C (Table) were infected with BCG at the same time as the first challenge with *T. pallidum* (see below). The BCG was inoculated into eight intradermal sites on the shaved back of each rabbit; 250 µg BCG (dry weight) was inoculated per site, a total of 2 mg per rabbit.

CHALLENGE WITH *T. PALLIDUM*

At one month

All rabbit groups, except E (Table), were challenged at eight intradermal shaved sites per rabbit with duplicate doses of 10⁴, 10³, 10², and 10 *T. pallidum* (see below for preparation). The BCG and *T. pallidum* were independently suspended in anaerobic bacterial medium (Graves *et al.*, 1975) and mixed together immediately before inoculation into the rabbit using the same syringe.

At six months

All rabbit groups (Table) were challenged at eight intradermal shaved sites per rabbit with duplicate doses of 10⁶, 10⁵, 10⁴, and 10³ *T. pallidum*.

MONITORING THE COURSE OF INFECTION

After the first challenge with *T. pallidum* rabbits were examined daily for one month, every two or three days thereafter for another month, and every

week thereafter for a further 2½ months, a total observation period of 4½ months. After the challenge with *T. pallidum* at six months, rabbits were observed for a total of 2 months, although no further lesions appeared after the first 3½ weeks. The rabbits were shaved regularly and the degree of induration was recorded. The appearance of any secondary syphilitic lesions was noted. The experimental rabbits were individually housed at 16-19°C and fed antibiotic-free rabbit pellets and water in unrestricted quantities. Only male rabbits were used.

PREPARATION OF CHALLENGE *T. PALLIDUM*

T. pallidum was propagated in the testes of male rabbits by inoculating 5 × 10⁷ viable *T. pallidum* per testis and harvesting it approximately 11 days later when a well developed orchitis was present. Elution of the *T. pallidum* from the minced orchitic testis required several sequential five-minute washings with 5-10 ml of sterile, prereduced anaerobic medium (Graves *et al.*, 1975). The washings were pooled under a flow of sterile oxygen-free nitrogen, counts were made in a Petroff-Hauser chamber, and the suspension was diluted to the required concentration for rabbit challenge.

SEROLOGICAL TESTING OF INFECTED RABBITS

To test for anti-cardiolipin (reagin) antibody the rapid plasma reagin (RPR) card test (Hynson, Wescott and Dunning, Baltimore) was used qualitatively. To test for anti-treponemal antibody the *T. pallidum* haemagglutination (TPHA) test (Fujizaki Pharmaceutical Co. Ltd., Shinjuku-ku, Tokyo) was used quantitatively. In the latter test titres of 1/80 or greater were considered to indicate antigenic stimulation with *T. pallidum*.

Results

SINGLE INFECTION WITH BCG (GROUPS B AND C)

At zero time

When rabbits in group B were challenged with *T. pallidum* (at one month) dermal syphilitic lesions appeared after incubation periods which were almost identical to those of control rabbits (group D). At the 10⁴ challenge sites lesions appeared after 14 days (control 13 days); at the 10³ challenge sites after 15 days (control 17 days); at the 10² challenge sites after 17 days (control 18 days); and at the 10 challenge site after 19 days (control 18 days). These incubation periods represent the mean of six lesions (two per rabbit for each of three rabbits). Thus BCG infection at zero time did not modify the incubation period of the subsequent syphilitic infection indicating that it

did not retard the growth of *T. pallidum* in these rabbits.

At one month

Rabbits in group C received their BCG infection at the same time and same site as their first challenge with *T. pallidum*. They did not receive BCG infection at zero time. Three days after infection they developed indurations of approximately 1 cm diameter at the sites of infection. These lesions faded after three to four weeks and disappeared by five weeks. All eight inoculation sites per rabbit showed similar indurated lesions, which appeared and disappeared together, unlike the control rabbits (group D) in which the sequential appearance of syphilitic lesions paralleled the size of the *T. pallidum* inoculum at that site. For this reason the lesions seemed to be due primarily to the growth of BCG, since each inoculation site received the same dose of BCG (250 µg). In two of the three rabbits in group C, the indurated lesions, after disappearing, reappeared again in a milder form at the original sites of inoculation about two months after the initial infection. They persisted for another two to four weeks before again disappearing. This further suggested that the indurations were due to BCG infection because *T. pallidum* lesions never reappear at the same site once they have regressed. Apparently the BCG growth masked any *T. pallidum* lesion development. The *T. pallidum* was not destroyed, however, since the rabbits subsequently developed syphilitic immunity. Furthermore, two of the three rabbits in group C did develop secondary, dermal, syphilitic lesions at sites on the shaved backs of the rabbits other than those inoculated. These appeared between day 36 and day 40 after inoculation, which is the normal time for secondary lesions to appear in *T. pallidum*-infected (but otherwise untreated) rabbits that do develop secondary lesions. The appearance of secondary lesions usually occurs in only a few rabbits after intradermal inoculation (Chesney and Schipper, 1950; Turner and Hollander, 1957).

DOUBLE INFECTION WITH BCG (GROUP A)

Rabbits in group A received two infections with BCG, one at zero time and another one month later. One to two days after the BCG infection at one month (and associated *T. pallidum* challenge) all eight inoculation sites showed strong delayed type hypersensitivity (DHS) reactions of equal intensity. However the induration did not fade, as would be the case with normal DHS, but remained strong (greater than 1 cm diameter) until about one month after infection. No sign of the classical syphilitic lesion (neither primary nor secondary) appeared on these rabbits, and, as before, the lesion seemed to be due

primarily to BCG growth or host hypersensitivity to BCG or both. Thus, *T. pallidum* growth was masked but evidently did occur because the rabbits subsequently developed syphilitic immunity.

CHALLENGE WITH *T. PALLIDUM* AT SIX MONTHS

After a second infection with *T. pallidum* at six months, all the rabbits of group A, B, C, and D failed to develop any syphilitic lesions whereas the control rabbits of group E all developed lesions with the following average incubation times: five days (10^6 inoculum); eight days (10^5 inoculum); 14 days (10^4 inoculum), and 21 days (10^3 inoculum). This demonstrates that the initial *T. pallidum* inoculation in all groups, including groups A and C where no primary syphilitic lesions were observed, did result in infection as shown by the subsequent development of immunity to repeat challenge.

SEROLOGICAL RESPONSES

Anti-cardiolipin antibody was synthesised by all rabbits in groups A, B, C, and D between one and two months after infection. Anti-treponemal antibodies (TPHA test) were also synthesised by all the above rabbits by two months after infection with *T. pallidum*. Titres did not vary significantly from one group to another.

Discussion

Mycobacterial species, especially BCG, and chemical fractions derived from mycobacteria have been widely used to enhance non-specific cell-mediated immunity to a variety of micro-organisms and tumours. In the case of bacteria it acts by antigen-induced blast transformation of specific T-lymphocytes and the release of lymphokines which activate normal host macrophages to develop enhanced intracellular killing ability. This latter ability is mainly non-specific (Mackaness, 1971).

Earlier work by Graves and Johnson (1975) demonstrated that BCG given intravenously one month before intradermal challenge with *T. pallidum* failed to modify the development of syphilitic lesions in the rabbit. The simultaneous administration of immune syphilitic serum markedly delayed the onset of lesions but there was no synergy evident between the immune serum and the BCG, suggesting that opsonisation had not occurred. Schell *et al.* (1975b) also reported that BCG failed to suppress a challenge with intravenous *T. pallidum* in rabbits while Baughn *et al.* (1977a) noted that the use of *Propionibacterium acnes* as a macrophage stimulant failed to modify the course of an infection with *T. pallidum* in rabbits even when given intradermally. Harris and

Thoen (1977) reported that the intradermal injection of BCG in mineral oil did protect rabbits from challenge with *T. pallidum* five days later. This finding is not consistent with the present observations, although differences in experimental procedure did occur.

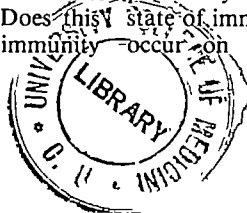
There is evidence that cell-mediated immunity does develop in the host (rabbit or man) during the course of an infection with *T. pallidum* (Musher *et al.*, 1976; Kiraly, 1976), and possibly it plays some role in the regression of lesions and the ultimate recovery of the host—for example, in human infections where recovery is sometimes spontaneous. Is phagocytosis by macrophages an important effector mechanism of cell-mediated immunity in syphilis? After *in-vitro* studies, Lukehart and Miller (1978) claim that it is and that immune serum significantly enhanced *in-vitro* phagocytosis of *T. pallidum* by rabbit peritoneal macrophages. If macrophages are important, why does BCG not enhance *T. pallidum* killing? Perhaps the activation of *T. pallidum*-killing macrophages is highly specific, requiring certain obligatory treponemal antigens, and cannot therefore be induced with BCG.

Alternatively, the macrophage may not play an important role in immunity. *T. pallidum* could be killed by some other mechanism, such as immune cytolysis involving specific T-lymphocytes or antibody-mediated K-cell killing. Electron microscopical observations of early syphilitic lesions have shown that very few *T. pallidum* are intracellular and that most are extracellular (Azar *et al.*, 1970; Lauderdale and Goldman, 1972). This, of course, does not rule out a role for phagocytic cells later in the infection (during the immune stage) but it does suggest that rapid and efficient uptake of *T. pallidum* by normal host cells does not occur as part of the initial response to infection. Assuming that the *T. pallidum* cell is not susceptible to phagocytosis in the normal host, it is perhaps not surprising that enhancing the intracellular killing power of the macrophages with BCG does not result in enhanced killing of *T. pallidum*. Wicker *et al.* (1977) reported that leucocytes (monocytes and polymorphonuclear leucocytes) from syphilitic rabbits showed an increased incidence of nitroblue tetrazolium (NBT) reduction. This increase was observed 10 days after infection and reached a maximum 30 days after infection with *T. pallidum*; the NBT-positive leucocytes returned to normal 50 days after infection. This corresponds to the period taken for the lesion to heal but not to the period of maximum immunity to reinfection. Rabbits are not usually fully resistant to *T. pallidum* challenge until about three months after infection (Turner and Hollander, 1957). Does this suggest that phagocytosis and induction of immunity occur on challenge the implication is that the

are temporally well separated? It is the immunologically more important macrophages that are the key to this question and not the polymorphonuclear leucocytes. Although human monocytes did not phagocytose virulent *T. pallidum in vitro* (Brause and Roberts, 1978), the role of the macrophage in the syphilitic lesion is still to be determined.

The inability of spleen cells from immune syphilitic rabbits to confer immunity to challenge (Baughn *et al.*, 1977b), even with inbred recipient rabbits, implies that lymphocytes (of either subclass T or B) are not effector cells in the immune animal. Antibodies are the best documented line of defence against *T. pallidum* in rabbits, possibly acting in concert with some cellular elements of the host, since alone they do ameliorate infections but without giving complete protection. Metzger and Smogor (1975) reported some degree of immunity following passive transfer of lymphocytes from immune rabbit lymphnodes, but this would have included B (antibody-producing) lymphocytes, so the effect observed may have been due to antibody synthesis in the recipient rabbit.

The present study has shown the inability of BCG, a commonly used reticuloendothelial system stimulant, to stimulate the rabbit's immune system against *T. pallidum*. The injection of BCG into the same intradermal sites as the *T. pallidum* did mask the development of syphilitic lesions due to the growth of, or hypersensitivity to, BCG or both. The normal method of determining if these rabbits were infected with *T. pallidum*, in the absence of observable, characteristic lesions, is to wait until several months after inoculation and then to transfer excised lymph nodes to the testes of a normal rabbit. This latter rabbit is then examined regularly for a syphilitic orchitis in which *T. pallidum* is demonstrable by darkfield microscopy (Miller, 1971). This method was not used in this study owing to a shortage of space in our laboratory and because an equally sound technique was available that did not require additional rabbits. This method is based on the observation that after an untreated syphilitic infection of about three months' duration these rabbits are refractory to challenge with the same strain of *T. pallidum*. Hence, when no lesions are observed a rabbit can be tested for the presence of infection by challenging with *T. pallidum* at least three months after the time of suspected infection. In this experiment a time period of five months was used to be certain that immunity had developed in the infected rabbits. If, on challenge, no lesions develop the rabbits can be assumed to have been previously infected, thus explaining their current state of immunity. If, on the other hand, lesions did occur on challenge the implication is that the



previous exposure to *T. pallidum* had not resulted in a syphilitic infection.

In the present study, all rabbits were shown to have been infected by the challenge with *T. pallidum* at one month by virtue of their immunity to the challenge with *T. pallidum* at six months. In support of this conclusion, all rabbits developed antibodies to cardiolipin (reagins) and *T. pallidum* antigens as shown by the RPR and the TPHA tests respectively. Although a serological response, as measured by the TPHA test, could have developed by simple antigenic stimulation and without an infection the development of cardiolipin antibodies represents tissue destruction caused by *T. pallidum* infection (Fredericksson *et al.*, 1968).

The author wishes to thank Ian McLean for excellent technical assistance. The work was supported by grants from the National Health and Medical Research Council (Australia), the Utah Foundation, the Ian Potter Foundation, the Danks Trust, Monash University and the estate of the late George Adams, from whom funds are gratefully acknowledged.

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Limited protection of rabbits against infection with *Treponema pallidum* by immune rabbit sera

S. GRAVES AND J. ALDEN

From the Department of Microbiology, Monash University Medical School, Alfred Hospital, Prahran, Australia

SUMMARY After intradermal infection of rabbits with 3×10^6 *Treponema pallidum* (Melbourne 1 strain) samples of serum were taken at one, two, three, four, and six months after infection. Normal rabbits were passively immunised with these sera, challenged with intradermal doses (10^4 , 10^3 , 10^2 , 10) of *T. pallidum*, and the latent periods of infection, lesion diameters, and the number of inoculation sites developing into lesions were observed. The sera taken at three, four, and six months reduced the number of intradermal inoculation sites that developed into syphilitic lesions after challenge with 10^4 *T. pallidum*. These same three sera also increased the latent period of infection after challenge with 10^4 *T. pallidum*. The transfer of 50 ml of immune serum per rabbit over a nine-day period before challenge had very little effect on the course of the challenge infection. Only a low level of immunity in rabbits to this strain of *T. pallidum* appears to be mediated by immune serum but this small degree of protection did increase with time after infection. Enhanced growth of *T. pallidum* in the serum-recipient rabbits did not occur, thus suggesting that none of the sera was immunosuppressive.

Introduction

It is generally recognised that immunity to *Treponema pallidum* in both humans and rabbits develops slowly (Turner and Hollander, 1957). Humans with untreated syphilis remain susceptible to reinfection until such time as their initial infection becomes latent (Magnuson *et al.*, 1956). Persons with primary and secondary lesions remain susceptible to exogenous *T. pallidum*. In rabbits the disease must progress for approximately three months before complete resistance to challenge is established (Turner and Hollander, 1957). The reasons for this slow onset of immunity are not known but may be due to the recently recognised immunosuppression that occurs early in syphilis (Pavia *et al.*, 1978). A circulating factor (Levene *et al.*, 1969) which decreases the ability of the host lymphocytes to be transformed appears to play a part. The immunosuppressive factor may be of bacterial origin,

resulting in 'antigenic overload' (Musher *et al.*, 1976), or of host origin—for example, an antibody (Medici, 1972). In either case, its concentration should theoretically rise soon after infection and then fall again as latency is established and the host becomes immune.

To investigate whether or not this occurs, samples of serum were taken at monthly intervals from infected rabbits until they became immune. These sera were used to immunise passively normal rabbits which were then challenged with *T. pallidum*; the course of infection was followed to see whether or not serum 'immunosuppressive' or serum 'protective' factors had been transferred.

The *T. pallidum* strain used in the study was isolated in 1977 from an anal chancre of a male homosexual in Melbourne, Australia. Since most recent work in the field of syphilis immunology has been carried out using the Nichols strain (Nichols, 1914), isolated many decades previously, we investigated the protective properties of immune rabbit serum raised against this current *T. pallidum* strain and compared it with information already available about immune serum against the Nichols strain.

Address for reprints: Dr S. Graves, Department of Microbiology, Monash University Medical School, Alfred Hospital, Prahran 3181, Australia

Received for publication 8 May 1979

Materials and methods

ISOLATION OF *TREPONEMA PALLIDUM* (MELBOURNE 1 STRAIN)

This strain of *T. pallidum* was isolated in February 1977 from a small anal lesion (positive by darkfield microscopy) of a male homosexual by inoculation of the exudate into a rabbit testis. After 4½ months the rabbit developed antitreponemal antibodies and by six months treponemes were observed by darkfield microscopy of an aspirate from the orchitic testis. The rabbit was killed and an eluate from a minced testis transferred to another rabbit by intratesticular inoculation and the strain maintained in this manner.

HARVEST OF *T. PALLIDUM*

After intratesticular inoculation of approximately 5×10^7 *T. pallidum* per testis orchitis developed in about two weeks. Orchidectomy with an aseptic technique was performed on the rabbit immediately after it had been killed by intravenous injection of 4 ml sodium pentobarbitone (200 mg/ml). The testes were minced in sterile anaerobic *T. pallidum* maintenance medium (Graves *et al.*, 1975) and the eluted *T. pallidum* adjusted to the required concentration. Counts were made in a bacterial counting chamber under darkfield microscopy. The eluted *T. pallidum* usually had a concentration of about 10^7 /ml. Dilutions were carried out under a stream of sterile, oxygen-free nitrogen.

EXPERIMENTAL INFECTION WITH *T. PALLIDUM*

Each of seven adult rabbits was inoculated intradermally into its shaved back with 3×10^6 *T. pallidum*. These rabbits were bled at monthly intervals to provide sera. Rabbits were housed at 18°C and fed antibiotic-free feed and water in unrestricted quantities.

SEROLOGICAL MONITORING

At monthly intervals antitreponemal antibody tests were performed on the individual sera and the pooled serum. Rapid plasma reagin (RPR) (Hynson, Wescott and Dunning, Baltimore) and *Treponema pallidum* haemagglutination (TPHA) tests (Fujizaki Pharmaceutical Co. Ltd., Shinjuku-ku, Tokyo) were performed according to the manufacturers' direction.

TRANSFER OF SERA

Sera taken each month from the infected rabbits were pooled and stored frozen until the final (six-month) samples were obtained. Each of the monthly pools were then sterilised by filtration through a 0.45 µm Millipore filter. Twenty-one adult male rabbits were selected as recipients of the serum and

divided into seven groups of three rabbits: group 1 received no serum; group 2, normal serum; group 3, one-month serum; group 4, two-month serum; group 5 (one rabbit died during administration of the serum), three-month serum; group 6, four-month serum; and group 7, six-month serum. Each rabbit received a total of 50 ml serum, given in five intravenous doses of 10 ml at nine, seven, five, and two days and two hours before challenge.

T. PALLIDUM CHALLENGE OF RECIPIENT RABBITS

Each rabbit had its back shaved and marked into a 10-cm × 10-cm grid of 16 squares. The challenge doses of *T. pallidum* used were 10^4 , 10^3 , 10^2 , and 10 , all given intradermally (i.d.) in quadruplicate. Rabbits were kept shaved and were examined daily for the appearance of syphilitic lesions at the inoculation sites. The first sign of a definite induration at the site of inoculation was taken as the end of the latent period for that particular inoculum. The diameter of the zone of induration of each syphilitic lesion was measured 28 days after challenge. Rabbits were examined daily for 34 days after challenge and again on days 42 and 68. No new lesions appeared after day 32. During the observation period rabbits were housed at 18°C as before.

TEST OF IMMUNE STATUS OF DONOR RABBITS

A few days after the six-month serum collection from the seven infected rabbits, each of these rabbits was challenged intradermally with 10^6 *T. pallidum* at a shaved site on the back. None of these inoculation sites developed into syphilitic lesions, indicating that rabbits donating serum six months after infection were immune to rechallenge—that is, the serum taken from the six-month donor rabbits was in fact from immune rabbits.

Results

The donor rabbits, infected with 3×10^6 *T. pallidum* i.d., developed positive TPHA and RPR titres by one month after infection, with the exception of one rabbit which gave a negative result to the RPR test. By two months, the TPHA titres had reached a maximum (Table 1). However the only observed effect of the one-month and two-month sera on the recipient rabbits (after transfer of the serum and challenge with *T. pallidum*) was to produce apparently smaller lesions compared with normal rabbit serum and then only after a 10^4 challenge-dose (data not presented). Anti-*T. pallidum* antibodies present within two months of infection appeared to have very little host-protective ability.

Table 1 *Syphilis serology on pooled rabbit sera taken up to six months after infection with T. pallidum (3 × 10⁶ i.d., Melbourne 1 strain)*

Time after <i>T. pallidum</i> infection (months)	Test results	
	RPR	TPHA titre
0 (preinfection)	—	<1/80
1	+	1/1280†
2	+	1/5120‡
3	+	1/5120§
4	+	1/5120§
6	+	1/5120§

+ Positive — negative

*One of seven rabbits still negative

†All seven rabbits positive

‡Range of titres from individual rabbits, 1/320-1/5120

§Range of titres from individual rabbits, 1/1280-1/5120

Serum taken three months after infection retarded the development of lesions after challenge with 10⁴ *T. pallidum* (Table 3) and reduced the number of inoculation sites that developed into syphilitic lesions after challenge with 10 *T. pallidum* (Table 2). It therefore had some protective effect.

Serum taken four months after infection reduced the size of the syphilitic lesions, retarded their development (increased latent period) after challenge with 10⁴ *T. pallidum* (Table 3), and reduced the

number of inoculation sites that developed into syphilitic lesions after challenge with 10 *T. pallidum* (Table 2). This serum also had some protective ability.

Serum taken six months after infection significantly reduced the number of inoculation sites that developed into syphilitic lesions after challenge with 10 *T. pallidum* (Table 2) and also retarded the development of lesions after challenge with 10⁴ *T. pallidum* (Table 3). It was the most protective of the sera tested. However none of the sera had any significant effect on 10³ and 10² *T. pallidum* challenge doses.

In general, none of the transferred sera was markedly successful in modifying the course of the *T. pallidum* infection. Depending on the criteria of protection used, different sera had varying (but minimal) protective ability. On the basis of the reduction in the number of inoculation sites developing into syphilitic lesions, the immune sera taken at three and six months were equally protective, while on the basis of the retardation of the development of syphilitic lesions, the immune sera taken at four and six months were equally protective.

Table 2 *Number of T. pallidum lesions (numerator) in serum-treated rabbits as a fraction of total intradermal inoculation sites (denominator) for four different T. pallidum (Melb. 1) inoculation doses after passive transfer of syphilitic rabbit sera*

Passive transfer to recipient rabbits (in months after <i>T. pallidum</i> infection)	<i>T. pallidum</i> lesions for four different <i>T. pallidum</i> inocula			
	Inoculation sites			
	10 ⁴	10 ³	10 ²	10
None	12/12	12/12	12/12	12/12
Normal rabbit serum	12/12	12/12	12/12	12/12
1	12/12	12/12	12/12	12/12
2	12/12	12/12	12/12	12/12
3	8/8	8/8	8/8	4/8
4	12/12	12/12	12/12	9/12
6	12/12	12/12	11/12	7/12

Table 3 *Latent period (days) for the development of syphilitic lesions in recipient rabbits after intradermal infection with graded doses of T. pallidum*

Passive transfer to recipient rabbits (in months after <i>T. pallidum</i> infection of donor rabbits)	Latent period (days) after four different <i>Treponema pallidum</i> challenge doses							
	10 ⁴		10 ³		10 ²		10	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
None	10.2	2.1	11.7	0.5	16.6	2.0	21.0	2.7
Normal rabbit serum	12.8	1.9	17.0	2.3	20.4	2.6	22.9	3.2
1	10.8	1.5	13.0	1.5	16.0	1.3	21.1	1.9
2	14.2	1.8	17.2	2.1	20.3	1.7	24.2	3.2
3	17.4	3.4*	18.5	2.4	22.2	3.5	24.5	1.7
4	16.2	1.2†	17.8	1.8	20.1	2.0	22.9	1.8
6	15.7	0.9†	17.7	1.6	19.9	2.7	24.7	3.6

*Significant difference ($P < 0.005$) by Student's *t* test when compared to normal serum transfer†Significant difference ($P < 0.001$) by Student's *t* test when compared to normal serum transfer

SD = Standard deviation

Discussion

Using the Nichols strain of *T. pallidum*, a number of investigators have already shown that the transfer of immune rabbit serum to normal rabbits, and their subsequent challenge, does not completely protect the recipient rabbits (Perine *et al.*, 1973; Sepetjian *et al.*, 1973; Turner *et al.*, 1973; Graves and Johnson, 1975; Bishop and Miller, 1976a; Weiser *et al.*, 1976). Depending on the dose of immune serum given and its time of administration in relation to the challenge with *T. pallidum*, the development of syphilitic lesions may be retarded (that is, they have longer latent periods), with atypical morphology (smaller and less indurated), and regress more quickly, possibly without ulceration. With very low challenge doses of *T. pallidum*, lesions may even be completely prevented.

In our experiment we transferred five lots of 10-ml volumes of serum per rabbit over a nine-day period, the last dose being two hours before challenge with *T. pallidum*. The rationale for this procedure was to ensure a high level of transferred serum proteins throughout the tissues of the recipient rabbits at the time of first exposure to introduced *T. pallidum*. Sequential addition of immune serum to normal rabbits has produced rising antibody titres (Sepetjian *et al.*, 1973).

Where a protective effect was observed immune serum may either kill some of the introduced challenge *T. pallidum* or (more likely) be bacteriostatic. Thus, as the passively introduced antibodies decay the treponemes start to grow and establish a (modified) infection. Antibody and complement are apparently not bactericidal *in vivo* unlike *in vitro* (TPI test, Nelson and Mayer, 1949). This may be because *in vitro* the *T. pallidum* cannot grow and maintain its structural integrity. A similar phenomenon has been reported with *Haemophilus influenzae* type b, which is resistant to antibodies in a medium supporting growth but is lysed by antibodies in a medium that does not support growth (Newman *et al.*, 1973).

It is now well recognised that immunity to *T. pallidum* is slow to develop in both humans (Chesney, 1927) and rabbits (Eberson, 1921; Turner and Hollander, 1957). Recently Bishop and Miller (1976b) detected some immunity in rabbits as early as 11 days after infection, but not until three months were all their rabbits completely resistant to challenge.

In our experiments the first two serum samples taken after rabbit infection with *T. pallidum* (at one and two months) apparently had some protective ability as shown by a statistically significant reduction in the size of the syphilitic lesions produced

by a challenge with 10^4 *T. pallidum*. However no similar protection was found after challenge with 10^3 , 10^2 , or 10 *T. pallidum* making this protective effect of doubtful significance. The sera taken at three, four, and six months after infection not only retarded the appearance of the syphilitic lesions after challenge with 10^4 *T. pallidum* but also reduced the number of inoculation sites developing into lesions after challenge with 10 *T. pallidum*. In addition the serum taken at four months also reduced the size of the lesion after challenge with 10^4 *T. pallidum*. These observations indicate that protective factors were gradually being produced by the *T. pallidum*-infected rabbits. There was no evidence that any serum contained an immunosuppressive factor, although if it were present at low levels it may have been masked by the protective factors which were probably antibodies. In general, the longer the period of infection in the rabbit the greater the degree of passive immunity conferred by serum from that rabbit, but immunity was never complete.

Possibly, if serum had been taken less than one month after infection, the presence of immunosuppressive factors in the serum could have been inferred should there have been more rapid growth of the challenge *T. pallidum* in the serum-recipient rabbits than in the control rabbits. Furthermore, the factor or factors responsible for early immunosuppression in syphilis may possibly be cell-bound.

Normal rabbit serum gave some level of protection against a challenge with *T. pallidum* (Table 3), and consequently all comparisons were made between transfer with immune and normal serum rather than with no serum. The presence of rabbit protein on the surface of the introduced *T. pallidum* (Logan 1974) may have resulted in a cross-reaction to allotypic differences in these proteins owing to the immunisation of the recipient rabbit with the transferred immune rabbit serum proteins nine days before challenge.

In this study a new, recently isolated strain of *T. pallidum* (Melbourne 1) was used. All recent experimental work in syphilis immunology has involved the use of the Nichols strain, which may be atypical. Nichols (1914) himself thought this was so. Other workers have also suggested that there is serological heterogeneity among *T. pallidum* (Eberson, 1921; Chesney, 1927; Turner and Hollander, 1957). Our observations on the Melbourne 1 strain suggest that the basis of immunity is only partially humoral and in this respect it does not differ from the Nichols strain.

In hamsters the closely related bacterium, *Treponema pertenue*, can be prevented from producing cutaneous lesions by the passive transfer of immune serum (Schell *et al.*, 1978) but, as in

syphilis, immune serum does not completely protect the host from infection.

The authors wish to thank Mr Ian McLean for excellent technical assistance. This work was supported by the National Health and Medical Research Council (Australia), the Utah Foundation, the Potter Foundation, the Danks Trust, the estate of the late George Adams, and Monash University, from whom financial assistance is gratefully acknowledged.

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Reverse passive haemagglutination test for the rapid identification of *Neisseria gonorrhoeae* and detection of penicillinase production

R. MUNRO AND R. MALLON

From the Department of Bacteriology, Institute of Clinical Pathology and Medical Research, Wentworthville, Sydney, Australia

SUMMARY In a comparison of the reverse passive haemagglutination test (RPHA) with the direct immunofluorescent and rapid carbohydrate utilisation tests for the identification of *Neisseria gonorrhoeae* isolated from clinical specimens, 315 isolates of oxidase-positive Gram-negative diplococci were tested as pure 24-hour-old subcultures and samples from 108 similar organisms were taken directly from primary isolation plates. A similar test system was used to detect penicillinase production. Results showed agreement in 97·8% of organisms tested with the RPHA and conventional methods for identification of *N. gonorrhoeae*; similarly there was good agreement with conventional methods for detection of penicillinase production. The test was reliable and could be read within four hours; a result was therefore available on the same day the clinical specimen was received. The time and work involved in identifying *N. gonorrhoeae* using the RPHA was less than with conventional methods, but differentiation between *N. gonorrhoeae* and other *Neisseria* species from throat swabs proved difficult.

Introduction

Although a presumptive diagnosis of gonorrhoea is generally made at a venereal disease clinic on the basis of the findings in a Gram-stained smear, it is important to supplement and confirm this diagnosis in the laboratory (Jephcott *et al.*, 1974). The laboratory must provide a rapid and definitive identification of *Neisseria gonorrhoeae* for this information to be of value to the clinician. At present we receive 10 000 specimens per year for the isolation and identification of *N. gonorrhoeae*. The primary isolation medium used is modified New York City medium (Young, 1978a, b), and oxidase-positive, Gram-negative diplococci on these plates are further identified using direct immunofluorescence (Difco). Any organism giving a negative result is identified from a 24-hour-old pure subculture using a rapid carbohydrate utilisation test (Young *et al.*, 1976). We find that approximately 83% of organisms can be

diagnosed by direct immunofluorescence from the primary isolation plate at 18-24 hours but 17% of organisms require two tests for identification; this delays the results reaching the clinician by 24 hours and also adds to the cost of identifying the organism. The rapid carbohydrate utilisation test is most reliable when done with pure cultures; we have not consistently been able to obtain accurate results using organisms from primary isolation plates.

In an attempt to find a single test which would identify *N. gonorrhoeae* within 24 hours of the clinical specimen being taken, we have evaluated a reverse passive haemagglutination test (RPHA). This test detects a macroscopic agglutination reaction between a suspension of lysed *N. gonorrhoeae* and human group O red cells sensitised with rabbit antigonococcal antibodies. The results of this test were compared with those of the direct immunofluorescence and rapid carbohydrate utilisation tests. A similar system using human red cells sensitised with rabbit anti-penicillinase antibodies was evaluated for the detection of penicillinase production. Results were compared with the acidometric method of detecting penicillinase production described by Slack *et al.* (1977).

Address for reprints: Dr R. Munro, Department of Bacteriology, Institute of Clinical Pathology and Medical Research, PO Box 60, Wentworthville, Sydney, New South Wales 2145, Australia

Received for publication 8 April 1979

Material and methods

Specimens were obtained from patients attending the Venereal Disease Clinic in Sydney. These were taken from the urethra and cervix in women and from the urethra, throat, and rectum in men. Swabs were inoculated on to modified New York City medium at the clinic, incubated at 36°C in candle jars, and transported to the laboratory the following day.

Initial evaluation of the RPHA was performed with 24-hour-old subcultures of suspected *N. gonorrhoeae*. At the end of that trial, a further 108 isolates were tested directly from the primary isolation plates. Organisms in the laboratory were incubated at 36°C in an atmosphere containing 10% CO₂.

REAGENTS

The reagents used (Abbott Laboratories) were:

- (1) Lyophilised anti-*N. gonorrhoeae* sensitised red cells (GONO-cells).
- (2) Lyophilised anti-penicillinase sensitised red cells (PENI-cells). A blue dye was incorporated to facilitate reading of the test.
- (3) Positive control (penicillinase-containing *N. gonorrhoeae* protoplasm). Preservative: thiomersal 1:10 000.
- (4) Negative control (*Neisseria perflava* protoplasm). Preservative: thiomersal 1:10 000.
- (5) Specimen dilution buffer (0.1 M phosphate buffer with protein and ion stabilisers). Preservative: 0.1% sodium azide.
- (6) Turbidity control (latex suspension adjusted to give an organism count of approximately 10⁶ organisms/ml.)
- (7) A 25 µl disposable dropper.
- (8) Lysing solution (4 mol/l sodium hydroxide).

All reagents were stored at 4°C. Lyophilised red cells were reconstituted with distilled water and had a life of two weeks when stored at 4°C.

PREPARATION OF SPECIMENS

Using a cotton-wool swab to facilitate mixing, colonies were removed from plates of modified New York City medium and an even suspension was made in 1 ml of injection-quality distilled water to match the turbidity control. The suspension was then lysed with 5 µl of 4 mol/l sodium hydroxide using a precision micropipette. Only specimens which achieved a clarity comparable with a distilled-water blank in one minute were subsequently tested. (In fact, lysis of specimens was easily achieved, and no *N. gonorrhoeae* so far tested has failed to lyse.) The lysed specimens were then left for 15 minutes before the test was performed.

PERFORMANCE OF ASSAYS

A positive control, negative control, and specimen dilution buffer control were included in each test run. The microtitre trays used contained round-bottomed wells (Kayline Product TUS 96), although for performance of PENI-cell assays a more readable result was obtained with V-shaped wells.

Twenty-five microlitres of specimen dilution buffer was added to the required number of wells. Thereafter 5 µl of lysed specimen, 5 µl of positive control, and 5 µl of negative control were added to the appropriate wells. When PENI-cell assays were also performed, all specimens were tested in duplicate. After being mixed, 25 µl of GONO-cell and PENI-cell suspensions were added to each well containing controls and unknown specimens. The plates were shaken for two minutes using a Cooke microshaker. They were then left for a minimum of four hours on a non-vibrating surface at room temperature. Results were read with the aid of a test reading mirror. The positive GONO-cell control exhibited a dispersed red-brown settling pattern with an irregular periphery, and test specimens showing this pattern were read as positive. The positive PENI-cell control showed a similar dispersed blue settling pattern, and test specimens showing this pattern were read as positive. A negative result consisted of a button of cells at the bottom of the well.

REPRODUCIBILITY

To ascertain the reproducibility of the test, one *N. gonorrhoeae* strain and one *Neisseria meningitidis* strain were selected and GONO-cell and PENI-cell assays performed on each isolate 25 times. These two organisms were then subcultured daily for eight days and GONO-cell and PENI-cell assays performed daily on the subcultures.

SPECIFICITY

Twenty-four-hour-old subcultures of six *N. gonorrhoeae* and six *N. meningitidis* colonies were assayed with GONO-cells and PENI-cells. Six assays were also performed using GONO-cells with 24-hour-old subcultures of *Staphylococcus aureus*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and a *Moraxella* strain.

Results

IDENTIFICATION OF *N. GONORRHOEAE*

The results obtained from clinical isolates of suspected gonococci are given in Tables 1 and 2. Discrepancies between conventional methods of identification and RPHA are summarised in Table 3. Detection of penicillinase production is summarised in Table 4 and compared with conventional methods.

Table 1 Results from 24-hour pure cultures of oxidase-positive, Gram-negative diplococci

Patients investigated		RPHA result			Identified by direct IF and RCUT		
Sex	Site	No.	+	-	<i>N. gonorrhoeae</i>	<i>N. meningitidis</i>	Other
M	Urethra	205	203	2	205	0	0
	Rectum	53	51	2	51	1	1
	Throat	7	7		4	3	0
	Total	265					
F	Urethra/cervix	48	48	0	48	0	0
	Rectum	1	1	0	1	0	0
	IUD	1	1	0	1	0	0
	Total	50					
Total M and F	All sites	315	311	4	310	4	1

+ Positive - negative

Direct IF = Direct immunofluorescence (Difco)

RCUT = Rapid carbohydrate utilisation test

RPHA = Reverse passive haemagglutination

IUD = Intrauterine device

Table 2 Results of oxidase-positive, Gram-negative diplococci from primary isolation plates

Patients investigated		RPHA result			Identified by direct IF and RCUT		
Sex	Site	No.	+	-	<i>N. gonorrhoeae</i>	<i>N. meningitidis</i>	Other
M	Urethra	69	69	0	69	0	0
	Rectum	17	17	0	17	0	0
	Throat	2	0		0	2	0
	Total	88					
F	Urethra/cervix	20	20	0	20	0	0
	Rectum	0	0	0	0	0	0
	Total	20					
Total M and F	All sites	108	106	2	106	2	0

+ Positive - negative

Direct IF = Direct immunofluorescence (Difco)

RCUT = Rapid carbohydrate utilisation test

RPHA = Reverse passive haemagglutination

Table 3 Discrepant results for RPHA from all isolates

Sex of patient	Site	RPHA results		
		No. of specimens	False-positive	False-negative
M	Urethra	274	0	2
	Rectum	70	2	2
	Throat	9	3	0
	Total	353	5	4
F	Urethra/cervix	68	0	0
	Rectum	1	0	0
	IUD	1	0	0
	Total	70	0	0
Total M and F	All sites	423	5 (1.2%)	4 (1%)

Table 4 Results of penicillinase production by RPHA and conventional methods

	Test results		
	+	-	Doubtful
PENI-cell	3	105	1
Conventional methods	3	106	0

Reproducibility and specificity

On repeated subculture of one *N. gonorrhoeae* strain and one *N. meningitidis* strain the GONO-cell and PENI-cell assays gave consistent results. The GONO-cell assay gave negative results with all organisms tested except *N. gonorrhoeae*.

There was no difference in the ability of RPHA to

identify *N. gonorrhoeae* from 24-hour pure subcultures and from primary isolation plates. Overall the RPHA gave a false-positive rate of 1.2% and a false-negative rate of 1% when compared with conventional methods. The false-positive results arose mainly from throat swabs containing *N. meningitidis* and, in one case, *N. catarrhalis*. It was noted during the study that several throat swabs contained both *N. meningitidis* and *N. gonorrhoeae*, and only careful scrutiny of isolation plates to identify both colonial types would prevent confusion in subsequent tests. The false-negative rate compared favourably with direct immunofluorescence suggesting that the rabbit anticonococcal antiserum used in the RPHA had a much broader spectrum of activity than that at present available in commercial immunofluorescent kits. The PENI-cell assay proved satisfactory for detecting penicillinase production.

Discussion

The RPHA has a number of advantages over the conventional methods we use at present for rapidly identifying *N. gonorrhoeae* and detecting penicillinase production. It enables a definitive diagnosis to be made the same day the clinical specimen is received (that is, after 18-24 hours incubation) in more cases than does direct immunofluorescence. The Venereal Disease Clinic thus has the result of culture by the time the patient is assessed at his follow-up visit. The main confusion with this test has occurred with throat swabs from which a high proportion of false-positive results was obtained. Since performing this trial, we have accumulated a further 13 throat swabs in which suspect *N. gonorrhoeae* was tested using the reverse passive haemagglutination test. All 13 swabs gave positive results but only four were confirmed as containing *N. gonorrhoeae* using conventional methods. Hence it is necessary to confirm the identity of *Neisseria* isolates from throat swabs with, for example, a rapid carbohydrate utilisation test.

The incubation time for this test is not critical and may be extended for up to 24 hours without affecting the result. Detection of penicillinase can be readily incorporated into the overall procedure. Since performing this trial, we have found a further eight organisms which produced penicillinase and all were satisfactorily identified by the RPHA test. These organisms had a minimum inhibitory concentration for penicillin of >2mg/l. The test takes approximately the same time to set up as direct immunofluorescence, the results are easier to read, and the test is less open to misinterpretation. A clear-cut end result means that fewer indeterminate reactions need confirming with further tests.

A comparison with an acidometric method for detecting penicillinase production showed that they were equally successful.

In conclusion we have found that the RPHA for rapid identification of *N. gonorrhoeae* and detection of penicillinase production from primary isolation plates has advantages over the conventional methods used at present. However, it does not distinguish well between *N. gonorrhoeae* and other *Neisseria*, and other tests are preferable for identifying *Neisseria* organisms from throat swabs.

We wish to thank Mr J. Williams, Abbott Laboratories, for providing us with reagents to perform these tests, and Dr Ann Walker, Venereal Disease Clinic, Sydney, for clinical specimens.

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Prevalence of rectal and pharyngeal infection in women with gonorrhoea in Sheffield

G. R. KINGHORN AND S. RASHID

From the Special Clinic, Royal Infirmary, Sheffield

SUMMARY The prevalence of gonococcal infection of the rectum and pharynx in 239 consecutive women with gonorrhoea presenting as named contacts was 47% and 10% respectively. In 2% of all patients the rectum and pharynx were each the sole sites of infection. Treatment failure occurred in three patients given a single dose of aqueous procaine penicillin 2.4 megaunits intramuscularly, two of whom had rectal infections; no treatment failures occurred in patients with pharyngeal infections.

The incidence of rectal gonorrhoea significantly increased with the duration of infection ($P < 0.001$). This suggests that autoinoculation from infected vaginal material is of major importance in the transmission of gonococcal infection to the rectum.

Introduction

The value of routine sampling of the rectum in women with suspected gonorrhoea attending venereal disease clinics in England was demonstrated by Bhattacharyya and Jephcott (1974). Despite further confirmatory reports (summarised by Klein *et al.*, 1977), routine investigations of rectal secretions in female contacts of men with gonorrhoea are carried out in only 35 (20%) of 173 clinics in England (Adler, 1978). Samples are taken routinely from the pharynx in even fewer clinics.

The present study aims to determine in those women with gonorrhoea who presented as known contacts (1) the prevalence of rectal and pharyngeal infection, and (2) if any relationship exists between the duration of infection and the rectal carriage of *Neisseria gonorrhoeae*.

Patients and methods

The study population consisted of 239 women with gonorrhoea attending this clinic as known contacts of gonorrhoea between 1 October 1977 and 31 September 1978.

SAMPLING

At the first visit swabs for Gram staining and culture were taken from each patient from the urethra,

cervix, and rectum and for culture only from the pharynx. If the first tests gave negative results they were repeated 4-6 days later. In most patients specimens from all four sites were again tested during follow up after treatment.

The methods of sampling from each site are summarised by Morton (1977). Rectal specimens were obtained with direct visualisation of the rectum (Bhattacharyya and Jephcott, 1974). The diagnostic criteria and cultural methods used are fully described by Jephcott and Rashid (1978).

TREATMENT

The standard treatment regimen was a single dose of aqueous procaine penicillin 2.4 megaunits i.m. Alternative regimens, given to those who were hypersensitive to penicillin, were spectinomycin 4 g i.m. or kanamycin 2 g i.m. Treatment failure was diagnosed if *N. gonorrhoeae* persisted on Gram-stained smears or culture swabs taken within one week of initial treatment in the absence of any further sexual contact.

DURATION OF INFECTION

The duration of infection, which was assessed from the clinical history, was taken as the number of days since initial contact with the presumed infected primary consort. Variation in the proportion of patients who had rectal infections for differing periods of time was assessed by the χ^2 test using Yates's modification. A 2×2 contingency table was constructed and the result given as one degree of freedom.

Address for reprints: Dr G. R. Kinghorn, Special Clinic, Royal Infirmary, Sheffield

Received for publication 18 April 1979

Results

Of the total 239 infections, 24 (10%) were diagnosed by Gram stain alone, 42 (18%) by culture alone, and 173 (72%) by both methods. The sites giving a positive result by either method are shown in Table 1. Two hundred and thirty-three (97%) infections gave a positive result by smear or culture or both at the first examination. A presumptive diagnosis of gonorrhoea was made for 184 (77%) infections by positive Gram-stain results from one or more of the three sites; this allowed treatment to be given at the patient's first visit.

Table 1 *Diagnosis of gonorrhoea in 239 infections affecting different sites by Gram-stained smear or culture or both*

Infections giving positive results from									
Cervix		Urethra		Rectum		Pharynx		Total	
No.	%	No.	%	No.	%	No.	%	No.	%
217	90.8	179	74.9	113	47.3	24	10	239	100

Gram-stained smears from the cervix and urethra gave more positive results than did those from the rectum (Table 2). However, 39% of all rectal infections gave positive results by Gram stain. In 58 (24%) infections, one site only gave a positive result (Table 3). The rectum or the pharynx were the only sites affected in 2% of all infections.

Two hundred and thirty infections were followed up at least once after treatment. Treatment failed in

Table 2 *Comparison of results by smear and culture of specimens from cervix, urethra, and rectum*

Diagnostic method		Results from					
		Cervix		Urethra		Rectum	
Smear	Culture	No.	%	No.	%	No.	%
+	-	25	11.5	15	8.4	4	3.5
-	+	53	24.4	62	34.6	69	61.1
+	+	139	64.1	102	57	40	35.4
Total		217	100	179	100	113	100

+ Positive - negative

Table 3 *Analysis of infections giving positive results from single site*

Diagnostic method	Positive results				
	Cervix	Urethra	Rectum	Pharynx	Total
Smear alone	12	4			16
Culture alone	11	4	3	4	22
Smear and culture	16	2	2		20
Total	39	10	5	4	58
% of all specimens	16.3	4.2	2.1	1.7	24.3

three of these after penicillin treatment; only one site was affected in each case, the cervix in one and the rectum in two. In all three instances results to cultures taken before and after treatment had been positive. No treatment failures occurred in the case of pharyngeal infections, even though all were treated with single-dose regimens.

The effect of duration of gonococcal infection on rectal carriage of *N. gonorrhoeae* is shown in Table 4. The results show that the frequency of rectal infection significantly increases with the duration of infection ($P < 0.001$).

Table 4 *Effect of duration of infection on incidence of rectal gonorrhoea*

Rectal culture result	Duration of infection (weeks)						Total	
	<1		1-2		>2			
	No.	%	No.	%	No.	%	No.	%
+	25	24.5	31	51.7	57	74	113	47.3
-	77	75.5	29	48.3	20	26	126	52.7
Total	102	100	60	100	77	100	239	100

+ Positive - negative
Fifty-six (34.6%) of 162 infections of less than two weeks' duration affected the rectum compared with 57 (74%) of 77 infections of over two weeks' duration ($\chi^2 = 31.0$; $P < 0.001$)

Discussion

The overall prevalence of 47% for rectal infections in female contacts with gonorrhoea and of 2% for rectal gonorrhoea alone compare with the mean prevalence rates of 44% and 4% respectively quoted by Klein *et al.* (1977). The finding of two out of three treatment failures for rectal infections alone in this study emphasises the need for routine rectal investigations in both the diagnosis and follow up of infected women.

The major mechanism of rectal infection is not clear. Ødegaard (1972) was unable to demonstrate an increased incidence of gonococcal proctitis with concomitant trichomoniasis. Dans (1975) found no difference in the admitted incidence of rectal intercourse in women with gonorrhoea between those with and those without rectal infection. He stated that peno-anal contact during foreplay was a more likely mechanism. Our finding that the incidence of rectal infection increased with longer duration of infection suggests that autoinoculation by infected vaginal secretions may be the major mechanism.

Pharyngeal gonorrhoea is usually asymptomatic, can be a source of metastatic disease, and may persist with or without treatment (Wiesner, 1975). The incidence of 10% in Sheffield in 1978 is similar to the 10% reported by Brø-Jorgensen and Jensen (1973) in Denmark, 11.3% reported by Ødegaard and Gundersen (1973) in Norway, and 10.3% reported

by Wiesner *et al.* (1975) in the USA. No treatment failures after single-dose regimens were found in this study. This is surprising. Ødegaard and Gundersen (1973) reported a failure rate of 48% of 51 women treated with ampicillin 2 g and probenecid 1 g. Wiesner *et al.* (1975), however, successfully treated 35 out of 36 patients with pharyngeal infections with aqueous procaine penicillin 4·8 megaunits. The low local incidence of gonococcal isolates that show relative resistance to penicillin (Jackson and Jephcott, 1975) may be a factor in our apparent success with a smaller, single dose of penicillin. It should be made clear, however, that most of our patients had a single test-of-cure culture swab taken from the pharynx, which may be unreliable (Brø-Jorgensen and Jensen, 1973).

Both rectal and pharyngeal infections are, therefore, shown to be common in women with gonorrhoea in Sheffield. The findings in this study support those who advocate the importance of performing routine rectal and pharyngeal cultures to establish the diagnosis and cure after treatment in women with suspected gonorrhoea.

We are grateful to the secretarial staff, for the typing of this manuscript, and to Dr R. S. Morton for his helpful guidance.

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Comparison of minocycline and ampicillin in gonococcal urethritis

M. A. WAUGH,* E. MARY COOKE,† B. B. G. NEHAUL,† AND JANET BRAYSON†

From the *Department of Genitourinary Medicine, Leeds General Infirmary, and the †Department of Microbiology, School of Medicine, University of Leeds

SUMMARY A prospective, randomised, single-blind trial was carried out to compare the efficacy and tolerability of minocycline and ampicillin in the treatment of gonorrhoea in men. One hundred and twenty men were treated with minocycline 300 mg and 121 men with ampicillin 2 g and probenecid 1 g. Cure rates were similar in both groups of patients. There were few side effects.

Of 135 strains of *Neisseria gonorrhoeae* tested to different concentrations of minocycline and penicillin, two were resistant to penicillin (minimum inhibitory concentration (MIC) $\geq 0.1 \mu\text{g/ml}$) and seven to minocycline (MIC $> 1.0 \mu\text{g/ml}$).

The incidence of PGU was 31% in those patients treated with minocycline and 34% in those treated with ampicillin plus probenecid, the difference not being statistically significant. PGU occurred more often after treatment with minocycline than in previous studies.

Introduction

Ampicillin has gained a place in the treatment of gonorrhoea since the early reports of Willcox (1963) and Alergant (1963). Used by itself there have been unacceptably high failure rates. Gundersen *et al.* (1969), as have others since, showed that the addition of probenecid, which blocks the renal excretion of penicillin causing a higher serum concentration, alleviated this problem. Adler (1978a) found that ampicillin was the most commonly prescribed antimicrobial for men with gonorrhoea in England and Wales, being used together with probenecid in one-third of clinics. It has no effect, however, when used as pivampicillin (Pondocillin) (an ampicillin derivative) on the development of PGU (Waugh and Nayyar, 1977). Minocycline hydrochloride, a semi-synthetic derivative of tetracycline (7-dimethylamine-6-deoxy-6-demethyl tetracycline hydrochloride), has been found to be of use in the treatment of gonorrhoea since the findings—albeit with differing results—of Pariser and Marino (1970) and Duncan *et al.* (1971). In the United Kingdom Shahidullah (1975) and Masterton and Schofield (1976) reporting its use

in the treatment of uncomplicated gonococcal urethritis in men found incidences of PGU of 11.8% and 5.1% respectively.

This study was carried out in 1977 and 1978 to compare (a) the efficacy and tolerability of minocycline and ampicillin in the treatment of uncomplicated urethral gonorrhea, and (b) the incidence of post-gonococcal urethritis (PGU) after treatment with these antimicrobials by means of a prospective, randomised, single-blind group comparative trial.

Patients and methods

Two hundred and forty-one men with acute gonococcal urethritis were randomly allocated to one of two single-dose treatment groups: group A received minocycline 300 mg; group B, ampicillin 2 g and probenecid 1 g taken with 210 ml of water in the presence of a nurse. The drugs were given according to a previously designed randomisation code in identical containers only identifiable by the patient's number.

In all cases microscopical findings were verified by bacteriological culture. For primary isolation and for subculture a modified Thayer-Martin medium was used. The constituents were Oxoid GC agar base, a chemical enrichment, 1% Isovitalex (Baltimore Biological Laboratories, BBL), and 10%

Address for reprints: Dr M. A. Waugh, Department of Genitourinary Medicine, General Infirmary, Great George Street, Leeds LS1 3EX

Received for publication 26 March 1979

defibrinated horse blood instead of haemoglobin. Antibodies (vancomycin, colistin, and nystatin) were not added. The fermentation medium was cystine-trypticase peptone agar (CTA medium, BBL) with added carbohydrates. During most of the trial cultures for *Chlamydia trachomatis* were not taken routinely from male patients.

FOLLOW UP

After treatment the patients were asked to return at seven, 14, and 28 days, although this showed much personal variation. At all follow-up visits the patient had a urethral smear and culture and a two-glass urine test performed. If after seven days more than five pus cells per high power field ($\times 100$ objective) were found in the urethral smear, or in shreds from centrifuged urine specimens, PGU was considered to be present. Patients were then treated with triple tetracycline (chlortetracycline hydrochloride 115.4 mg, tetracycline hydrochloride 115.4 mg, demeclocycline hydrochloride 69.2 mg; Deteclo) one tablet twice daily for 14 days. The patient was clinically cured if his discharge and other symptoms of urethritis had disappeared and if *Neisseria gonorrhoeae* was not found on microscopy or culture.

On each return visit, the patient was asked if he had noticed any side effects of treatment and if he had had coitus recently.

Any patient who was allergic to penicillin or tetracycline, was a habitual defaulter, was suspected of having syphilis, or had been given antimicrobials in the previous two weeks was excluded.

AGE, NATIONALITY AND PREVIOUS STD

The mean age, standard deviation (years), nationalities, and previous history of STD of the two groups are shown in Table 1. The symptoms and signs of dysuria and discharge for the two groups are given in Table 2.

DEFAULT RATE

Of 241 patients in the trial, 199 (82.6%) attended at least once after treatment. The duration of follow up is shown in Table 3.

SENSITIVITY TESTS

At the start of the trial 108 strains were overgrown with contaminants. A total of 135 strains were tested. Each isolate was confirmed as *N. gonorrhoeae*. Each strain was tested against both minocycline and penicillin. For each strain, isolated colonies from a 24-hour growth on modified Thayer-Martin medium without antibiotic were suspended in 1 ml of trypticase soy broth (BBL) until a faintly turbid suspension was obtained. Using a calibrated loop,

Table 1 Details of patient groups

	Group A (n = 120)	Group B (n = 121)
Mean age (SD)	27.2 (9.3)	26.4 (8.3)
Country of origin		
UK and Eire	77	75
West Indies	24	26
Others	17	16
Total	118*	117†
Previous history of STD (%)	48 (40)	41 (34)

*Two patients were reinfected during the trial

†Four patients were reinfected during the trial

Table 2 Symptoms and signs in patient groups

	Group A			Group B		
	No.	%	Mean duration (days)	No.	%	Mean duration (days)
Dysuria	78	65	3.7 $\pm$ 1.8 SD	79	65	3.8 $\pm$ 1.8 SD
Discharge	114	95	5.1 $\pm$ 2.4	115	95	5.8 $\pm$ 2.3

Table 3 Duration of follow up in each treatment group

Group	Duration of follow up (weeks)				
	0	1	2	4	Total
A	20	32	29	39	120
B	22	28	29	42	121

1.0 μ l of the suspension was placed on the plate of diagnostic sensitivity test agar (Oxoid) with 7% lysed horse blood containing varying concentrations of penicillin and minocycline (0.06-1.0 μ g/ml).

Plates were incubated for 48 hours in CO₂ and the end point—that is, the minimum inhibitory concentration (MIC)—of each strain was taken as that concentration of antibiotic which resulted in no growth of the strain.

The Oxford staphylococcus was used as the control on each plate containing antibiotic.

Results

Recurrences in the two groups are shown in Table 4. A further analysis of total cure (no PGU or positive results to smears or cultures) is shown in Table 5.

REINFECTION AND FAILURES

Group A

In the period of four weeks after treatment, there were two treatment failures; a further two patients had probably been reinfected.

Group B

In this group, there were three treatment failures

Table 4 Results of treatment

Group	No. of patients			% negative follow up and without PGU*	Treatment failures		
	Treated	Followed up	Cured		No.	%	Reinfected†
A	120	100	65	54/120	2	1.6/120	2
B	121	99	58	48/121	3	2.5/121	4

*% of total patients entered

†Based on all available clinical evidence and perusal of sexual partners' case histories

Table 5 Analysis of total cure*

Group	Follow up (weeks)			Total
	1	2	4	
A	26	21	18	65
B	22	19	17	58

*Cure means no PGU and negative results to smears and cultures

(including one case of β -lactamase-producing *N. gonorrhoeae*). Four patients had probably been reinfected.

No correlation of sensitivities and treatment failures was made during the trial. However, we considered it of paramount importance to break the code of the case with β -lactamase-producing *N. gonorrhoeae*. The patient was a 38-year-old Ghanaian postgraduate student found to have treatment failure at seven days and was subsequently cured with spectinomycin 2 g. He had no contacts in England and had left his wife in Accra, but her treatment could not be ascertained. The diagnosis was verified by Dr A. E. Wilkinson (Venereal Disease Reference Laboratory, London) on 29 June 1978 and reported to the World Health Organisation.

POST-GONOCOCCAL URETHRITIS

In group A, PGU occurred in 31 of 100 patients followed up. In group B it occurred in 34 (34.3%) of 99 patients followed up; the difference was not significant ($\chi^2 = 0.12$; $P > 0.5$, Yates's modification).

SIDE EFFECTS

Group A

One patient reported vomiting after medication but on follow up was found to be cured.

Group B

One patient developed a widespread purpuric eruption after 48 hours and was found to have glandular fever; one other developed candidal balanoposthitis within a week of treatment.

RESULTS OF SENSITIVITY TESTS

These are shown in Table 6.

Penicillin

Strains which were considered to be fully sensitive had an MIC of $<0.1 \mu\text{g/ml}$, those with a reduced

Table 6 Minimum inhibitory concentrations of minocycline and penicillin for 135 isolates of *Neisseria gonorrhoeae*

MIC of minocycline ($\mu\text{g/ml}$)	No. of strains with MIC of penicillin ($\mu\text{g/ml}$)							Total
	0.06	0.12	0.25	0.5	1.0	>1.0		
0.06	6	1	0	0	0	0		7
0.12	35	3	1	0	0	0		39
0.25	33	10	3	0	0	0		46
0.5	17	3	4	1	0	1		26
1.0	7	1	0	1	0	1		10
>1.0	2	1	2	2	0	0		7
Total	100	19	10	4	0	2		135

sensitivity an MIC of $0.1-0.5 \mu\text{g/ml}$, and those resistant an MIC of $>0.5 \mu\text{g/ml}$. One hundred strains were fully sensitive, 33 had a reduced sensitivity, and two were resistant.

Minocycline

Fully sensitive strains had an MIC of $<0.5 \mu\text{g/ml}$, those with a reduced sensitivity an MIC of $0.5-1.0 \mu\text{g/ml}$, and those resistant an MIC of $>1.0 \mu\text{g/ml}$. Ninety-two strains were fully sensitive, 36 had a reduced sensitivity, and seven were resistant.

Discussion

Both minocycline and ampicillin with probenecid were found to be effective forms of treatment, there being no significant difference in the outcome in either group.

There were minimal side effects and, as a result of careful history taking, no allergic reactions, although the well-known clinical picture of ampicillin rash in glandular fever was seen.

The default rate of 42 (17.4%) in 241 patients illustrates the importance of therapy that will be rapidly successful. It is probable that those patients who did not return were cured.

PGU rates (31% for minocycline and 34.3% for ampicillin with probenecid) were higher than in a previous trial (Waugh and Nayyar, 1977), in which 33 (23.4%) of 141 men treated with pivampicillin 1400 mg with probenecid 1g had PGU. The diagnostic criterion was then more than 10 pus cells per high power field. Again the PGU rate of 31% for minocycline was much higher than that of 11.8%

reported by Shahidullah (1975) and that of 5.1% reported by Masterton and Schofield (1976). The observation of Adler (1978b) that there is no single criterion for diagnosing non-gonococcal urethritis is aptly illustrated when an attempt to compare PGU rates from different trials is made. A single dose of minocycline appears to have no effect on the prevention of PGU. The similarity of the PGU rates with both drug regimens, using the same criteria, strengthens this supposition.

We should like to thank Messrs Lederle Limited for supplying drugs used in this clinical trial.

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Chlamydia trachomatis genital infections

F. D. GHADIRIAN AND H. G. ROBSON

From the Division of Infectious Diseases, Departments of Medicine and Microbiology, Royal Victoria Hospital and McGill University, Montreal, Quebec, Canada

SUMMARY The prevalence and clinical features of *Chlamydia trachomatis* infection were studied in men with nongonococcal urethritis (NGU), in their female sexual partners, and in other women. Of 92 patients with NGU, 36 (39%) were chlamydia-positive; although all had symptoms of urethritis, fewer than half had evident discharge. Clinical features of chlamydia-positive and chlamydia-negative NGU were indistinguishable. Most female contacts of chlamydia-positive patients with NGU were infected with *C. trachomatis*; contacts of chlamydia-negative patients with NGU seldom harboured chlamydia. Inflammatory and erosive changes were equally common in the uterine cervix of chlamydia-positive and chlamydia-negative women, although almost half of infected cases had no clinical signs of infection and no specific symptoms. In such women the present unavailability of sensitive screening techniques is a major obstacle to the effective control of chlamydial infection.

Introduction

Increasing emphasis is being placed on the role of *Chlamydia trachomatis* as a human pathogen, especially in infection of the genital tract. In men, *C. trachomatis* has been identified as an important cause of nongonococcal urethritis (NGU) (Dunlop *et al.*, 1972; Holmes *et al.*, 1975) and of ascending genital infections such as epididymitis (Harnisch *et al.*, 1977).

Although *C. trachomatis* has been isolated with increased frequency from the cervix of female sexual partners of men with chlamydia-positive NGU (Oriel *et al.*, 1972; Holmes *et al.*, 1975), its role as a pathogen in women is less clearly defined. Recent studies have provided evidence that infection may lead to cervical abnormalities (Swanson *et al.*, 1975; Rees *et al.*, 1977; Oriel *et al.*, 1978) and to salpingitis (Mårdh *et al.*, 1977).

Nongonococcal urethritis is not a notifiable disease in our community and there are no accurate statistics as to its prevalence. Experience in our clinic suggests that the disease occurs at least as often as gonococcal urethritis. The present study was undertaken to determine the prevalence of *C. trachomatis* infection in men attending our outpatient clinic with symptoms of nongonococcal urethritis, in their female contacts, and in women who presented for examination to exclude sexually transmitted diseases. We also

attempted to define the symptoms and abnormal clinical findings of our patients in relation to chlamydial isolation.

Patients and methods

SELECTION OF CASES

The study population was composed of patients who attended the Infectious Disease Clinic of the Royal Victoria Hospital between 18 November 1977 and 2 May 1978. The male patients included in the study were those with complaints of urethral discharge or dysuria but with negative results to Gram-stained urethral smears and to cultures for *Neisseria gonorrhoeae* on presentation at the clinic. Some men had been treated for culture-positive gonococcal urethritis within the preceding 28 days. Female sexual partners of some of the male patients in the study were included as were some women who attended clinic for screening examinations.

Chlamydial isolations were attempted only from patients who had not received antibiotic therapy within the preceding four weeks, except for penicillin treatment of gonorrhoea. The latter patients had been treated with single-dose regimens of aqueous procaine penicillin G 4.8 megaunits intramuscularly or ampicillin 3.5 g orally, each with probenecid 1 g orally.

CLINICAL EVALUATION

A standard medical history was obtained from each patient; in addition information on sexual behaviour

Address for reprints: Dr H. G. Robson, 3775 University Street, Montreal, Quebec H3A 2B4, Canada

and previous episodes of sexually transmitted disease was requested. Examination of male genitals included inspection of the penis and inguinal regions as well as gentle milking of the urethra in those patients who had no frank discharge. In most cases a two-glass urine test was performed. The criteria of Holmes *et al.* (1975) for diagnosing NGU were used: the presence of pyuria in the first 10 ml of voided urine, and the presence of 20 or more leucocytes in at least one of five randomly chosen microscopic fields at $\times 400$ magnification.

Female patients underwent genital examination which included inspection of the cervix after removal of adherent secretions. Abnormalities such as the presence of vaginal discharge, cervical erosions, inflamed or friable cervical epithelium, and hypertrophic nodular or cystic changes in the epithelium surrounding the cervical os were recorded.

COLLECTION OF SPECIMENS

Material for isolation of *C. trachomatis* was obtained with calcium-alginate swabs passed into the anterior urethra of men or endocervical canal of women. Swab tips were cut into plastic vials containing 2SPS transport medium (Darougar *et al.*, 1972). This material was kept at 4°C for up to four hours after collection and then stored at -70°C until inoculation into cell culture. Specimens for culture for *N. gonorrhoeae* were also obtained from the male urethra and from the cervix and rectum of female patients.

CELL CULTURE

The McCoy cell line was kindly provided by Dr S. Darougar (Institute of Ophthalmology, London). Confluent monolayers of cells in tissue culture flasks were trypsinised and suspended in CMA (Darougar *et al.*, 1971). One millilitre of cell suspension adjusted to contain 1×10^5 cells/ml was added to each of a series of flat-bottomed tubes containing a 13-mm diameter coverslip. After 48-72 hours' further incubation at 35°C confluent monolayers were ready for inoculation of specimens.

ISOLATION OF *C. TRACHOMATIS*

Specimens were rapidly thawed, agitated vigorously, diluted 1/2 with CMGA (Gordon *et al.*, 1969), and then inoculated in duplicate in flat-bottomed tubes from which the CMA had been removed. The inoculated tubes were centrifuged at $15\,000 \times g$ for one hour at 35°C. After a further two hours' incubation at 35°C the inoculum was replaced with fresh CMGA containing cycloheximide 1 µg/ml and the tubes were incubated for a further 72 hours. At that time the coverslips were Giemsa-stained and

examined by darkfield microscopy at $\times 200$ magnification for the presence of characteristic inclusions.

STATISTICS

Yates's χ^2 test was used for statistical evaluations.

Results

DEMOGRAPHIC AND CLINICAL FINDINGS

Men with NGU

Ninety-two male patients were included in the study. Of these, 36 (39%) were found to be infected with *C. trachomatis*. No features either in the history or on clinical examination were found to be useful in distinguishing culture-positive from culture-negative patients (Table 1). Urethral discharge could be

Table 1 Demographic and clinical features in male patients with NGU

Feature	Male patients			
	Chlamydia + (n=36)		Chlamydia - (n=56)	
	No.	%	No.	%
Mean age (years)	27.5		26.6	
Duration of symptoms (days)	16		16.4	
With first episode of urethritis	11	32.3	21	37.5
History of penicillin treatment (within 28 days)	14	38.8	25	44.6
<i>N. gonorrhoeae</i> -positive (within 28 days)	4	11.1	6	10.7
No. of sexual contacts within previous year				
1	4	11.1	12	21.4
2-4	18	50	25	44.7
>5	12	33.3	19	33.9
Not recorded	2	5.6	0	
With urethral discharge				
Purulent	8	22.2	13	23.2
Non-purulent	8	22.2	13	23.2
None	20	55.6	30	53.6
With pyuria				
Present	25	69.4	41	73.2
Absent	10	27.8	10	17.9
Not recorded	1	2.8	5	8.9

+ Positive - negative

objectively documented in 44% of chlamydia-positive and of 46% of chlamydia-negative patients. When present, the urethral discharge was equally often described as purulent or non-purulent. Although most patients had pyuria, 28% of men with positive culture results had negative results to the two-glass urine test. Thirty-two and 38% of culture-positive and culture-negative patients respectively stated that they had not previously had urethritis. The age of the patients, duration of their symptoms, number of sexual contacts in the preceding year; and

history of penicillin treatment within the preceding 28 days did not differ significantly between the two groups. Two (5.5%) of 36 chlamydia-positive patients and seven (12.5%) of 56 culture-negative patients claimed to engage solely in homosexual relationships.

Female patients

Eighteen of 45 women studied were infected with *C. trachomatis* (Table 2). The mean age of those infected was similar to that of the culture-negative group. Certain features such as the use of oral contraceptives, more than one sexual partner in the preceding year, and a concurrently positive culture result for *N. gonorrhoeae* appeared to be more frequent in the chlamydia-positive group; however none of the differences was statistically significant ($P>0.05$ in all instances).

Table 2 Demographic features in female patients with and without chlamydial infection

Feature	Female patients			
	Chlamydia + (n = 18)		Chlamydia - (n = 27)	
	No.	%	No.	%
Mean age (years)	25.2		25.5	
Using oral contraceptives	10	55.6	11	40.7
With concurrent gonococcal infection	5	27.8	3	11.1
No. of sexual contacts (within preceding year)				
1	6	33.3	14	51.9
2-4	9	50	11	40.7
>5	3	16.7	2	7.4
With vaginal discharge	9	50	17	62.9
With dysuria	1	5.6	4	14.8

+ Positive - negative

Cervical abnormalities were detected in both chlamydia-positive and chlamydia-negative patients (Table 3). The presence of inflammation, erosion, nodular or cystic changes, and friability of the cervical epithelium did not differ significantly between the two groups. It was striking that nearly half (44%) of patients shown to be infected with *C. trachomatis* had an entirely healthy cervix on clinical examination.

Female partners of infected men

We obtained specimens for culture from 11 contacts of men known to be infected with *C. trachomatis* (Table 4). Of these women, seven were chlamydia-positive while two others were found retrospectively to have taken a course of tetracycline within the preceding month. Of 10 contacts of chlamydia-negative men, only one was infected. This difference was significant ($\chi^2_1 = 4.80$, $P<0.05$).

Table 3 Abnormalities of cervix in relation to chlamydial infection

Abnormality	Female patients			
	Chlamydia + (n = 18)		Chlamydia - (n = 27)	
	No.	%	No.	%
None	8	44.4	13	48.1
Inflammation	7	38.9	10	37.0
Erosion	4	22.2	4	14.8
Friability	6	33.3	5	18.5
Nodular or cystic changes	3	16.7	3	11.1

+ Positive - negative

Table 4 Chlamydial infection in female contacts of men with NGU

	Female contacts		
	No.	No. examined	No. chlamydia +
Male patients			
Chlamydia-positive	36	11*	7
Chlamydia-negative	56	10	1

+ Positive

*Two patients had previously received tetracycline therapy

Discussion

The isolation rate of 39% for *C. trachomatis* from men with NGU is in agreement with the results of many other studies (Dunlop *et al.*, 1972; Oriol *et al.*, 1972; Holmes *et al.*, 1975; Schacter *et al.*, 1975). Some have reported isolation rates of over 50% (Perroud and Miedzybrodzka, 1978; Terho, 1978). Our patients were relatively unselected; we required only that they had complained of dysuria and urethral discharge and had negative culture results for *N. gonorrhoeae*. Since our clinic operated on only two mornings a week we found it impractical to recall patients for repeat examinations and cultures after overnight urinary continence. This may be the reason we could not record the presence of urethral discharge on clinical examination in most (50/92) patients in the study, and may explain why pyuria was absent in the first-glass urine of 10 of 36 chlamydia-positive men. Examining patients under less than ideal conditions did not appear to impair our ability to recover chlamydia from their urethral cultures.

Several authors have shown a correlation between the severity of urethral discharge and an increased isolation rate for *C. trachomatis* (Schachter *et al.*, 1975; Alani *et al.*, 1977; Terho, 1978). This correlation was not observed in our patients, as 16/42 (38%) with, and 20/50 (40%) without, visible discharge were chlamydia-positive. Furthermore, we were unable to confirm any relationship between the duration of symptoms and the rate of recovery of *C.*

trachomatis described by Holmes *et al.* (1975) and Alani *et al.* (1977).

Retrospective analysis of the histories and clinical findings in our male patients showed no clinically important differences between those with NGU who were infected with *C. trachomatis* and those who were not, as shown in several other studies (Oriel *et al.*, 1972; Holmes *et al.*, 1975; Perroud and Miedzybrodzka, 1978). This also poses a serious problem in the assessment of female patients. Numerous authors have described abnormalities of the uterine cervix in chlamydia-positive patients such as erosions, follicular and cystic lesions, and purulent cervical secretions (Dunlop *et al.*, 1972; Hilton *et al.*, 1974; Oriel *et al.*, 1974). Rees *et al.* (1977) have stated that hypertrophic cervical erosions and mucopurulent cervical discharge occur with increased frequency in chlamydia-positive patients. However, such abnormalities are not specific to chlamydial infection but may also be caused by *Trichomonas vaginalis* (Oriel *et al.*, 1978) and possibly by other agents. Although the number of female patients studied by us was small we could not distinguish on clinical grounds those who were infected with *C. trachomatis* from those who were not. Moreover, we found that many of our chlamydia-positive female patients had no complaints and appeared to be entirely healthy on clinical examination. This finding is certainly not unique to our study but again underlines the existence of a large infectious pool which is difficult to detect and is capable of perpetuating infection in the population. The application of serological procedures for population screening as described by Trehan *et al.*, (1978) may increase our ability to detect and control infection by *C. trachomatis*.

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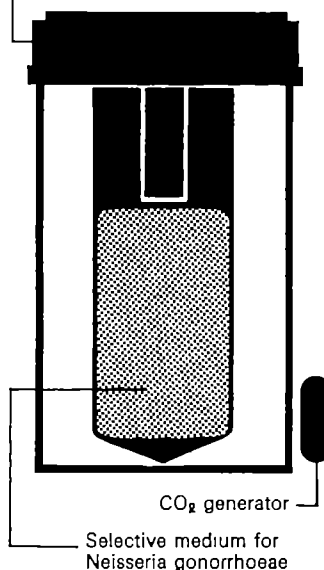
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Antichlamydial antibodies in pelvic inflammatory disease

P. D. SIMMONS,* T. FORSEY,† R. N. THIN,* J. D. TREHARNE,†
S. DAROUGAR,† F. LANGLET,* AND R. K. PANDHI*

From the *Department of Genital Medicine, St Bartholomew's Hospital, and the †Virus Laboratory, Institute of Ophthalmology, Judd Street, London

SUMMARY The role of *Chlamydia trachomatis* in pelvic inflammatory disease (PID) diagnosed without laparoscopy was assessed by measuring antichlamydial antibodies in the patient's serum and by comparing the results with those in patients with uncomplicated non-specific genital infection (NSGI) and gonorrhoea and in non-infected controls. A modified micro-immunofluorescence test was used.

Patients with severe PID had significantly more positive antichlamydial IgG and IgM results than did control subjects, patients with gonorrhoea, and patients with NSGI. Less severe PID was associated with significantly raised levels of antichlamydial IgG antibodies compared with NSGI and controls and with raised levels of IgM antibodies compared with controls. Two patients with PID had lower genital tract gonorrhoea, one of whom had raised antichlamydial antibody levels. These findings may indicate a mixed infection and therapy should be reviewed in such patients. A serological diagnosis of chlamydial infection is relatively easy and cheap and enables a rapid diagnosis of chlamydial infection to be made.

Introduction

Chlamydia trachomatis is associated with non-specific genital infection (NSGI) in women (Alani *et al.*, 1977), and causes acute salpingitis in some patients (Mårdh *et al.*, 1977). Chlamydial serum antibody levels correlate with *C. trachomatis* isolation from the cervix (Treharne *et al.*, 1978) and from the Fallopian tubes in patients with salpingitis diagnosed by laparoscopy (Treharne *et al.*, 1979). Unfortunately we, as well as others, do not have access to the type of laparoscopy service described by Treharne *et al.* (1979). However, when investigating causes of pelvic inflammatory disease (PID) we considered it useful to assess the serum chlamydial antibody levels in patients with PID diagnosed on clinical grounds. These were compared with the levels of antichlamydial antibodies in the serum of patients with uncomplicated genital infections and of controls.

Patients and method

Patients attending the Departments of Genital Medicine at St Bartholomew's Hospital and the Eastern Hospital, London, who were thought to have PID were included in this study. Criteria for the diagnosis of PID were based on those of Forslin *et al.* (1978) as follows:

- (A) Lower abdominal pain,
- (B) Pelvic tenderness with or without pelvic mass,
- (1) Vaginal discharge,
- (2) Menstrual irregularities,
- (3) Dysuria,
- (4) Dyspareunia,
- (5) Temperature $\geq 38^{\circ}\text{C}$,
- (6) ESR ≥ 15 /hour (Westergren),
- (7) White cell count $10\,000$ cu/mm ($10 \times 10^9/\text{l}$).

Patients were divided into two main groups: group 1 had strict admission criteria—that is, (A) + (B) + 2 of (1)–(7)—whereas group 2 had less strict criteria—that is, (A) + (B) + 1 of (1)–(7). Patients in group 1 were automatically included in group 2.

All patients were investigated for sexually transmitted diseases. Material from the lateral vaginal fornices was examined microscopically for

Address for reprints: Dr R. N. Thin, Department of Genital Medicine, St Bartholomew's Hospital, London EC1

Received for publication 16 June 1979

yeast cells and hyphae of *Candida* species after staining by Gram's method; secretion from the posterior fornix, mixed with saline, was examined microscopically for *Trichomonas vaginalis*. Vaginal material was inoculated on to Sabouraud's medium for culture for *Candida* species, and into Oxoid No. 2 trichomonas medium and examined after 48 hours' incubation. Specimens from the urethral meatus and cervical canal, stained by Gram's method, were examined for leucocytes and Gram-negative diplococci, and plain swabs from these sites were placed in Amies's modification of Stuart's transport medium. Within 24 hours they were plated on to lysed blood agar containing vancomycin, colistin, trimethoprim, and natamycin (Riddell and Buck, 1970). *Neisseria gonorrhoeae* was identified by sugar fermentation reactions using serum-free medium (Flynn and Waitkins, 1972). Specimens from the rectum, taken through a proctoscope, were also examined when gonorrhoea was particularly suspected. Cervical material was collected with an Ayre's spatula, wet-fixed in alcohol and ether, stained by Papanicolaou's method, and examined microscopically for malignant cells and inflammatory changes (Thin *et al.*, 1975). Blood was taken for syphilis antibody tests using the Venereal Disease Research Laboratory (VDRL) and *Treponema pallidum* haemagglutination (TPHA) tests. Cultures for *Chlamydia trachomatis* were not taken routinely.

Blood was taken for a modified micro-immunofluorescence test for chlamydial antibodies (Treharne *et al.*, 1977) on the day of diagnosis; the serum was separated and stored at -20°C until tested. Patients thought to have PID were matched for age, marital state, and country of origin with patients who had NSGI and with control women who had no evidence of genital infection or known contact with any sexually transmitted disease (STD). The diagnosis in patients with NSGI had been made on the basis of their contact with a man with non-specific urethritis or of the presence of excess urethral and cervical leucocytes in the absence of any pathogen (Treharne *et al.*, 1978). A group of patients with uncomplicated genitoretal gonorrhoea was also included, but unfortunately these could not be matched with the study patients.

Statistical comparisons were made using the χ^2 test with Yates's corrections.

Results

A total of 109 patients were included: 30 with PID (17 in group 1 and 13 in group 2), 30 with NSGI, 19 with gonorrhoea, and 30 as control subjects. The presence and levels of antichlamydial IgG and IgM

antibodies are shown in Tables 1 and 2. If antichlamydial IgG antibody at a level of 1/64 or higher and antichlamydial IgM at a level of 1/8 or higher in the blood is considered to suggest active chlamydial infection (Treharne *et al.*, 1979), patients with PID in group 1 had significantly more positive results than control subjects ($\chi^2 = 4.48$, $P < 0.05$) and patients with NSGI ($\chi^2 = 8.60$, $P < 0.01$) and gonorrhoea ($\chi^2 = 3.91$, $P < 0.05$) (Table 2). Patients in group 2 (including those with less strict criteria for PID) had significantly raised levels of antichlamydial IgG compared with control subjects ($\chi^2 = 5.69$, $P < 0.02$) and those with NSGI ($\chi^2 = 4.18$, $P < 0.05$), and significantly raised levels of antichlamydial IgM compared with control subjects ($\chi^2 = 4.01$, $P < 0.05$). Antichlamydial IgM antibody at a level of 1/8 or higher was present in 29-30% of patients with PID in groups 1 and 2. One patient in group 1 had genital gonorrhoea with raised levels of antichlamydial IgG and IgM, and two patients in group 2 had genital gonorrhoea, one with raised antichlamydial IgG and IgM levels and the other with no detectable antichlamydial antibodies. All the other patients with PID had negative results for gonococcal investigations and so were considered to have NSGI. In group 1 three patients had *T. vaginalis* and three yeasts; in group 2 five patients had yeasts, three *T. vaginalis*, and one cervical herpes virus type 2.

Table 1 Levels of antichlamydial IgG and IgM antibodies

Patient group	Antichlamydial antibodies (titre)					
	IgG ($\geq 1/64$)		IgM ($\geq 1/8$)		Total*	
	No.	%	No.	%	No.	%
With PID						
Group 1	10/17	59	5/17	29	10/17	59
Group 2	12/30	40	9/30	30	14/30	47
With NSGI	4/30	13	14/30	47	15/30	50
With gonorrhoea	4/19	21	5/19	26	7/19	38
Control	3/30	10	2/30	7	4/30	13

*With positive antichlamydial antibodies (IgG $\geq 1/64$ or IgM $\geq 1/8$)

Table 2 Levels of antichlamydial IgG antibody

Patient group	No. of patients	Levels of antichlamydial IgG antibody				
		1/16	1/32	1/64	1/128	1/256/1/512
With PID						
Group 1	17	2	2	3	2	3
Group 2	30	7	3	3	2	4
With NSGI	30	12	1	3	0	1
With gonorrhoea	19	8	0	3	0	1
Control	30	5	0	3	0	0

Discussion

Patients who attend departments of genitourinary medicine with evidence of PID, and in whom no evidence of gonorrhoea is found, are usually treated with tetracyclines for non-specific salpingitis. Our results suggest that this form of antibacterial treatment is reasonable as 59% of patients with PID in group 1 had raised serum antichlamydial IgG levels and 30% raised IgM antibody levels. Only one patient had gonorrhoea, and she also had raised antichlamydial IgG and IgM levels. When PID was, however, more loosely defined, only 40% of patients had raised antichlamydial IgG levels but still 30% had raised IgM levels. In this group, two patients had gonorrhoea, but only one had raised antichlamydial IgG and IgM antibody levels. Although some patients had *T. vaginalis* infection in the lower genital tract, it rarely appears to cause PID (Mårdh and Westrom, 1970). We found higher levels of antichlamydial IgM in patients diagnosed as having uncomplicated NSGI than in other categories, supporting our previous findings (Trehanne *et al.*, 1978).

Since in our practice the presentation of PID is usually acute and the patients need antibacterial treatment on the day of consultation, rapid diagnosis is essential. Gonorrhoea can be quickly excluded by Gram-staining and culture, but at present *C. trachomatis* cultures may take time, are not widely available, and are expensive. Serodiagnosis of chlamydial infection, which can be performed readily, rapidly, and relatively cheaply (Trehanne *et al.*, 1978) therefore has great advantages. Furthermore, it is useful in clinics which have no nearby facilities for *C. trachomatis* culture as serum is easily transported; pilot studies have shown that even small amounts of blood (0.1 ml) soaked on to cellulose sponges give reliable antibody results (Darougar *et al.*, 1978).

If antichlamydial antibody levels are raised in patients with PID and lower genital tract gonorrhoea, the therapeutic regimen should be reviewed, as these results may indicate a mixed infection.

We are grateful to Dr M. A. E. Symonds for allowing us to examine patients under his care. We thank Mrs Jane Wadsworth of the Computing Unit for Medical Sciences, St Bartholomew's Hospital, for her help with statistical analysis.

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Chlamydia trachomatis infection of the Fallopian tubes

Histological findings in two patients

B. R. MØLLER, L. WESTRÖM, S. AHRONS, K. T. RIPA, L. SVENSSON,
C. VON MECKLENBURG, H. HENRIKSON, AND P.-A. MÅRDH

From the Institute of Medical Microbiology, University of Aarhus; the Surgical University Clinic, Aarhus Amtssygehus, Denmark; the Institute of Medical Microbiology, the Department of Zoology, University of Lund, the Department of Gynaecology and Obstetrics, and the Department of Pathology, University Hospital, Lund, Sweden

SUMMARY In two patients with acute salpingitis, *C. trachomatis* was isolated from the cervix. In one of the patients, the organism was also recovered from the Fallopian tubes, and in the other, chlamydial inclusions were found in Giemsa-stained tubal epithelial cells. A significant change in micro-immunofluorescence antibodies to *C. trachomatis* occurred in both patients during the course of the disease. The Fallopian tubes of both patients were removed and studied by conventional histological techniques and, in the case of one of them, by transmission electron microscopy.

Introduction

Chlamydia trachomatis is a common cause of sexually transmitted genital infections (Schachter, 1978). It can be recovered from the cervix of about one third of women with signs of cervicitis but from only a small percentage of healthy women (Hilton *et al.*, 1974; Oriel *et al.*, 1978; Ripa *et al.*, 1978).

In patients with laparoscopically verified acute salpingitis, *C. trachomatis* has been isolated from the cervix in 19 of 53 cases and from six of 20 tubal specimens from these patients (Mårdh *et al.*, 1977). Evidence of chlamydial infection in women with acute salpingitis has also been demonstrated by serological means (Treharne *et al.*, 1979).

In non-human primates experimental salpingitis with *C. trachomatis* has been reported (Ripa *et al.*, 1979).

In the present study two patients with acute salpingitis with evidence of tubal infection with *C. trachomatis* are reported. In both patients the tubes were removed and studied by histological techniques, and, in one of them, by transmission electron microscopy.

Patients and methods

SAMPLING TECHNIQUES

Specimens for the isolation of *C. trachomatis*, mycoplasmas, and ureaplasmas were obtained by rotating a sterile, cotton-tipped swab in the cervical canal and in the lumen of the uterine tubes. Specimens for isolation of gonococci were taken from the cervix, the urethra, and from the rectum with a cotton-tipped swab treated with charcoal.

TRANSPORT MEDIA

Specimens for the isolation of chlamydia were transported and stored at -20°C in a sucrose-phosphate buffer supplemented with $50\text{ }\mu\text{g}$ gentamicin and $2.5\text{ }\mu\text{g}$ amphotericin B per ml (Gordon *et al.*, 1969). The specimens for the isolation of mycoplasmas were transported in liquid B medium (Freundt *et al.*, 1979), and those for ureaplasmas in liquid S medium (Freundt *et al.*, 1979). The *Neisseria gonorrhoeae* specimens were delivered to the laboratory in a modified Stuart's medium (Gästrin *et al.*, 1968).

ISOLATION PROCEDURES

C. trachomatis was cultured using cycloheximide-treated McCoy cells (Ripa and Mårdh, 1977). The culture techniques for mycoplasmas, ureaplasmas (Freundt *et al.*, 1979) and *N. gonorrhoeae* (Mårdh

Address for reprints: Dr B. Møller, Institute of Medical Microbiology, Bartholin Building, University of Aarhus, DK-8000 Aarhus C, Denmark

Received for publication 27 April 1979

and Weström, 1970) have been described in detail elsewhere.

SEROLOGICAL STUDIES

Serum antibodies to *C. trachomatis* were studied using a micro-immunofluorescence test (Treharne *et al.*, 1977). Fluorescein isothiocyanate conjugated rabbit anti-human anti-IgG and anti-IgM globulins (Dakopatt A/S, Copenhagen, Denmark) were used. The sera were titrated in two-fold dilutions; the lowest dilution used was 1/8.

Determination of antibodies to mycoplasmas and ureaplasmas was performed using an indirect haemagglutination test according to the method of Krogsgaard-Jensen (1971). Gonococcal anti-pilar antibodies were determined by an indirect haemagglutination test (Reiman and Lind, 1977).

HISTOLOGICAL TECHNIQUES

The tissue specimens were fixed in 10% formalin and processed by routine histological methods, including staining with haematoxylin and eosin, and with Giemsa.

TRANSMISSION ELECTRON MICROSCOPY

The specimens were fixed in 2.5% glutaraldehyde in

0.2 mmol/l cacodylate buffer, pH 7.4, for two hours. After repeated rinsing in the buffer, the specimens were refixed in 2% osmium tetroxide using the same buffer, dehydrated in ethanol and embedded in Vestopal W. Sections, 1 µm thick, and were examined by phase contrast microscopy. Ultrathin sections were then cut on an LKA-Ultrathome, contrasted with lead citrate and uranyl acetate, and viewed in a Zeiss EM 10 electron microscope.

CASE REPORTS

Case 1

A 27-year-old white African woman (gravid 4, para 2, legal abortion 1) was referred for legal abortion in the tenth week of pregnancy. On bimanual palpation the uterus was enlarged, corresponding to the tenth week of pregnancy. No sign of genital infection was detected. Erythrocyte sedimentation rate (ESR) was 2 mm/h and white blood cell count (WBC) $11.0 \times 10^9/l$ with a normal differential count.

On 10 April 1978, legal abortion was performed by dilatation and suction curettage under general anaesthesia as an outpatient. No immediate complications were noted.

Two weeks later the patient was readmitted with low abdominal pain of three to four days' duration. General examination was normal. A moderate amount of brownish discharge was found in the vagina. The uterus was of

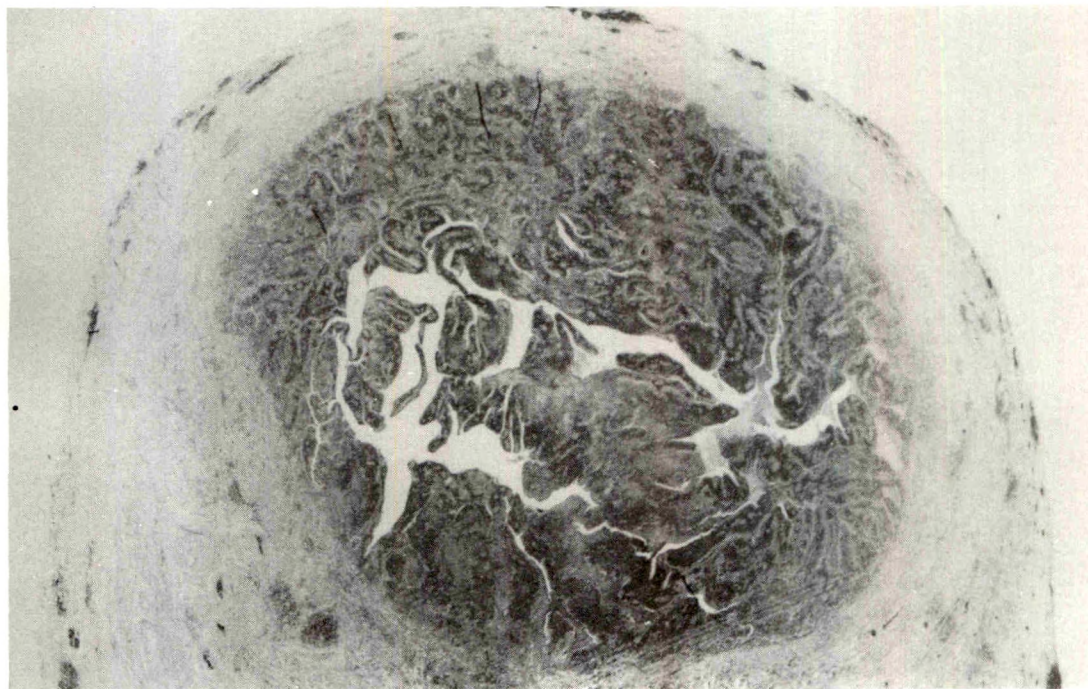


Fig. 1 Cross-section of Fallopian tube showing oedema and an intense infiltration of the epithelium, the subepithelial connective tissue, the muscular layer, and extending into the subserous layer (Case 1). Stained with haematoxylin and eosin, $\times 40$.

normal size and no adnexal masses were palpated. The pelvic examination caused moderate pain. Rectal temperature was 37.5°C, ESR 28 mm/h, and WBC $14.0 \times 10^9/l$. A clinical diagnosis of salpingitis was made, and the patient was treated in hospital with penicillin 10⁶ IU and streptomycin 500 mg twice daily for six days.

During the following three weeks the rectal temperature fluctuated between 37.5°C and 38.5°C. ESR increased to 64 mm/h. On repeated pelvic examinations increasing tenderness was noted and, therefore, 26 days after admission a laparotomy was performed. At operation both Fallopian tubes were swollen, about 5 cm in diameter. A purulent exudate poured from the mouth of the oviduct. On the right side there was a tubo-ovarian abscess while the left ovary was normal. Bilateral salpingectomy was performed. Ampicillin 2 g and metronidazole 1.2 g a day were given daily for six days postoperatively. The postoperative course was uneventful and the patient was discharged eight days after the operation.

Histological examination of the tubal specimens showed a marked inflammatory infiltrate through all the layers of the uterine tubes (Fig. 1). The lumina were filled with an exudate containing lymphocytes and desquamated epithelial cells. The epithelium, which was injured in some areas, was oedematous and infiltrated with inflammatory cells (Fig. 2). The muscle layers were infiltrated with

numerous lymphocytes and some polymorphonuclear leucocytes. The cellular infiltration extended into the subserous layer.

Giemsa-stained specimens examined by darkfield illumination showed intracytoplasmic inclusions in surface epithelial cells. The appearance of these inclusions was consistent with those found in *C. trachomatis* infected cells.

Cultures for gonococci and mycoplasmas from the tubes and cervix gave negative results. *U. urealyticum* was isolated from the cervix but not from the uterine tubes. *C. trachomatis* was recovered from the cervix the day before legal abortion was performed. At laparotomy five weeks later we failed to isolate *C. trachomatis* from the tubes. No chlamydial IgM or IgG antibodies were detected in serum samples taken the day before the therapeutic abortion. On day 41 after the abortion, the IgM micro-immunofluorescence antibody titre was 1/128 and that of IgG 1/32. Two months later the IgG titre was 1/128 while no IgM antibodies could be determined.

No IHA antibodies to mycoplasmas and ureaplasmas and no anti-pilar antibodies to gonococci were found before the abortion nor during the follow-up period.

Case 2

A 31-year-old Caucasian woman of Polish origin (gravida



Fig. 2 Section of Fallopian tube showing the epithelium and the subepithelial tissue infiltrated with inflammatory cells. The epithelium is injured in some areas. The lumen contains inflammatory cells and desquamated epithelial cells (Case 1). Stained with hematoxylin and eosin, $\times 200$.

6, para 2, legal abortion 4) applied for legal sterilisation in January 1978 and was admitted on 20 April for the operation. After menstruating she had observed a yellowish discharge. Three days before admission she developed low abdominal pain. On admission her rectal temperature was 37.6°C, and ESR 40 mm/h. Vaginal examination showed a small amount of yellowish discharge with an equal number of vaginal epithelial cells and leucocytes in the wet smear. The uterus was of normal size but there were bilateral masses about 5 cm in diameter. The patient experienced moderate pain on pelvic examination.

A preoperative diagnosis of bilateral tubo-ovarian abscesses was made and, because the patient had applied for sterilisation, laparotomy was performed 26 hours after admission. No antibiotic treatment had been given before laparotomy.

At operation both Fallopian tubes were swollen, about 5 cm in diameter, with pus in the mouth of the oviduct. The tubal wall was about 5-10 mm thick. The ovaries were partly covered with a purulent discharge but no peritonitis was present. A bilateral salpingectomy was performed and doxycycline 200 mg was administered intravenously during the first 24 hours followed by 100 mg by mouth daily.

The postoperative course was uneventful and the patient left hospital on the sixth day after operation.

Histological examination of the tubes showed marked inflammatory changes through all layers of the tubes. The mucosal lining was very oedematous with inflammatory cells in the submucous and muscular layers to the serous lining. The lumen of the tube was full of leucocytes (Fig. 3).

By transmission electron microscopy a specimen from the epithelium of the Fallopian tubes was from one to several cells thick. There were ciliated and non-ciliated cells, both of which had surface microvilli and goblet cells at different stages of maturity lying interspersed among the ciliated cells and containing numerous vesicles filled with mucus. The cytoplasm of the goblet cells contained a granular endoplasmic reticulum. The nucleus of the ciliated and the non-ciliated cells varied in shape from round to oval or were greatly elongated. The nuclear membrane was often indented; the cytoplasm of these cells contained mainly agranular endoplasmic reticulum. The mitochondria, mainly oblong in form, were sometimes situated in the apical part of the cell. There were also lysosomes of different size both in the ciliated and non-ciliated cells. The apical part of these cells often protruded into the lumen (Fig. 4).

Cultures for gonococci gave a negative result. *C. trachomatis* was isolated from the cervix and the Fallopian tubes. Sera collected on day 21 postoperatively contained

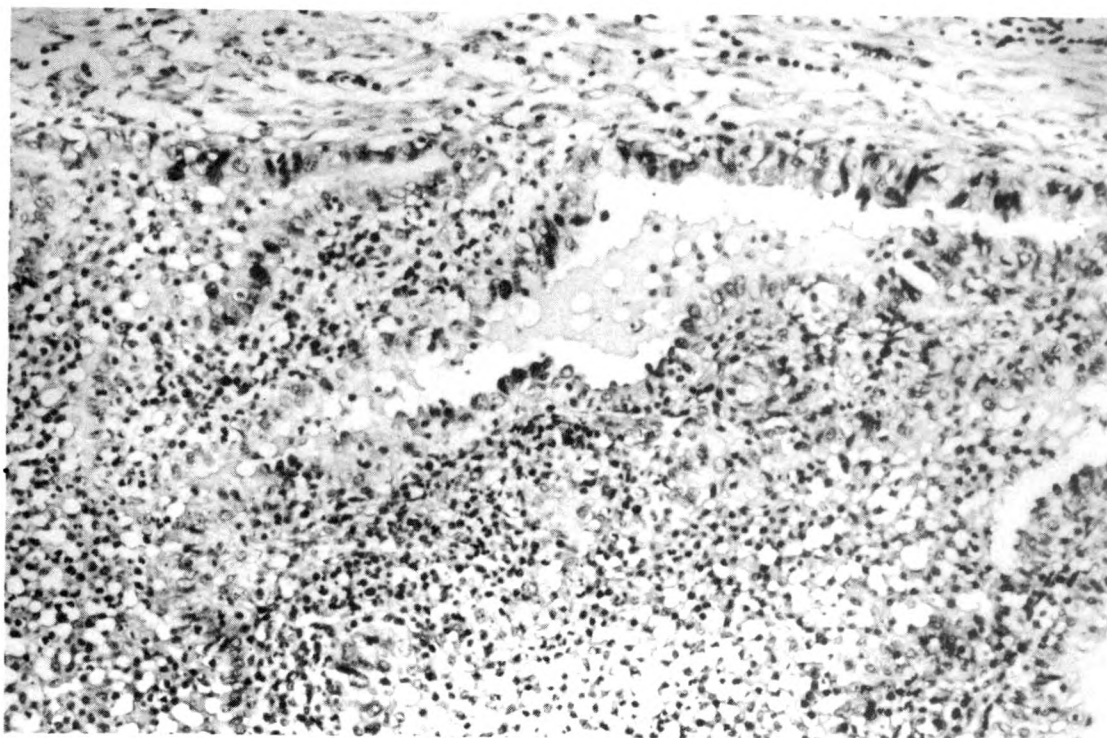


Fig. 3. Section of Fallopian tube. Marked inflammatory changes are evident throughout all the layers of the tube. The lumen contains leucocytes. The epithelium is oedematous and injured in some areas. Marked oedema with inflammatory cells in the submucous and muscular layers can be seen (Case 2). Stained with hematoxylin and eosin, $\times 200$.

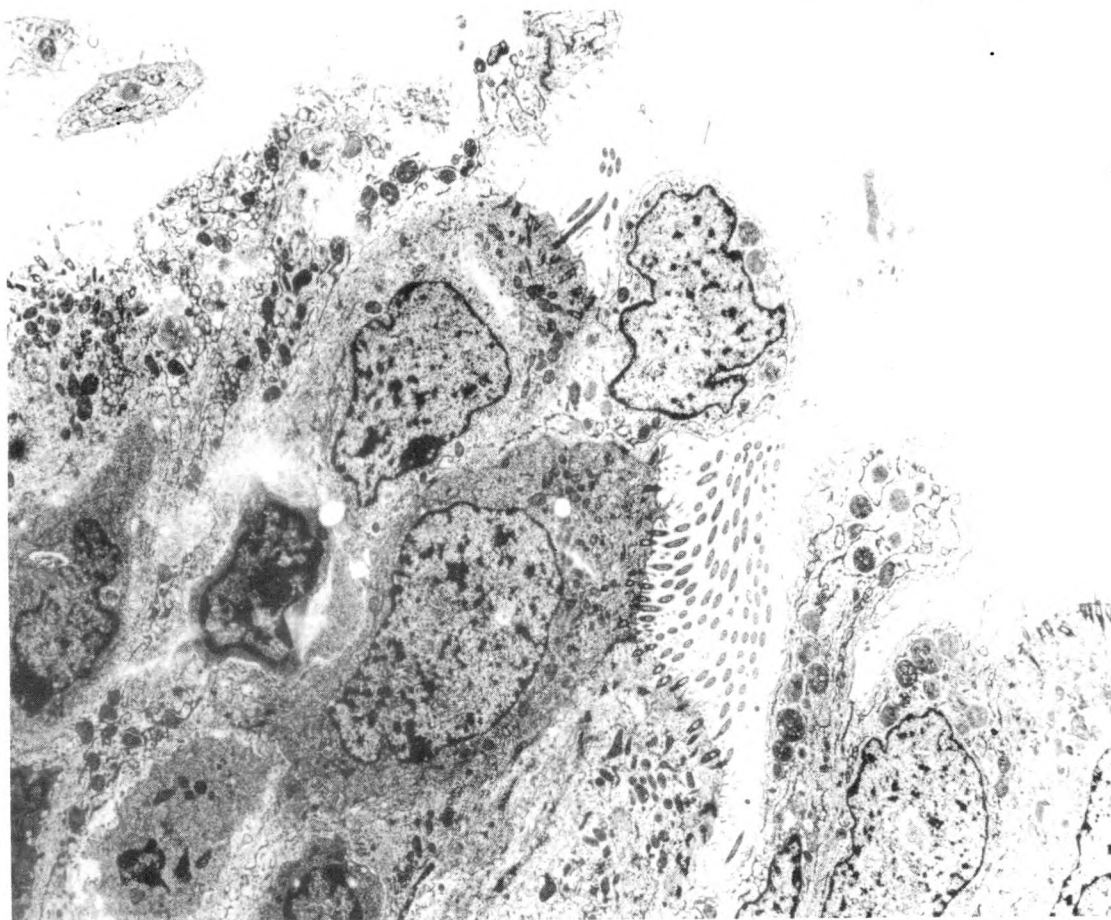


Fig. 4 Transmission electron micrograph of the surface epithelium of a Fallopian tube of a patient (Case 2) with acute salpingitis with evidence of *Chlamydia trachomatis* infection, $\times 4700$.

no IgM micro-immunofluorescence antibodies to *C. trachomatis* but IgG antibodies at a titre of 1/128. A second serum specimen was collected after a further month and contained IgG antibodies at a titre of 1/16 while no IgM antibodies were found. No anti-pilar antibodies to gonococci were found in the sera tested.

Discussion

Two main groups of non-tuberculous salpingitis have been recognised (Novak and Woodruff, 1974). In gonococcal salpingitis, the micro-organisms are spread from the lower genital tract through the lumen of cervical canal via the uterine mucosa to the tubes, which become red and swollen and contain a purulent exudate. Microscopically, the epithelium and the subepithelial tissue show an intense inflammation. In some areas the luminal surface epithelium is destroyed. Adhesion of the mucosal

folds are often seen. In nongonococcal salpingitis, the causative micro-organism is believed to gain entrance to the tissue through lesions in the cervical or endometrial epithelium and to spread to the parametria and tubes via blood vessels and lymphatics. The inflammatory swelling, which affects both the parametria and the tubes, is more pronounced than in gonococcal salpingitis. The swelling of the tubes is due to an oedematous thickening of the tubal wall. The tubal epithelium is usually intact and there is no exudate in the lumen.

Gonococci are seldom isolated from tubal specimens in women with acute salpingitis and cervical gonorrhoea (Mårdh *et al.*, 1978). The prognosis for fertility in antibiotic-treated cases of salpingitis with gonorrhoea is significantly better than in cases of salpingitis in which gonococci cannot be recovered (Weström and Mårdh, 1977). During the 1970s, the annual number of cases of

salpingitis with gonorrhoea in our hospital catchment region has been declining, even though the total number of such cases of acute salpingitis has increased during the same period (Weström and Mårdh, 1977). There is evidence that salpingitis caused by *C. trachomatis* is now more common in our area than that due to gonorrhoea (Mårdh *et al.*, 1977).

Experimental *C. trachomatis* salpingitis in grivet monkeys causes histological changes in the tubes which are similar to those of gonococcal salpingitis in man (Ripa *et al.*, 1979). *Mycoplasma hominis* produces changes which are similar to those in patients with nongonococcal salpingitis (Weström and Mårdh, 1977).

In Case 1, *C. trachomatis* was isolated from the cervix before abortion, at which time no antibodies to the micro-organism were detected. Two weeks after this operation, the patient was admitted to hospital with signs of acute salpingitis. She was treated with penicillin and streptomycin, which had no effect on the clinical course. Laparotomy was performed and both tubes were found to be swollen. Bilateral salpingectomy was performed. The histological changes in the tubes were the same as those described in textbooks for gonococcal salpingitis. Immunofluorescent studies showed intracytoplasmic chlamydial inclusions in some of the cells. IgM and IgG micro-immunofluorescence antibody titres to *C. trachomatis* were significantly raised.

Gonococci, mycoplasmas, and ureaplasmas could not be recovered from the tubes. The patient did not develop antibodies to any of these micro-organisms during the course of the disease. As in Case 1, therapeutic abortion may enable *C. trachomatis* to spread by way of the cervical canal to the tubes.

The other patient was admitted three days after the start of low abdominal pain. No diagnostic procedures had been performed before admission. When salpingitis was diagnosed, bilateral salpingectomy was performed as the patient had asked for legal sterilisation. Chlamydia were isolated from the Fallopian tubes and from the cervix whereas gonococci were not. During the postoperative period, a significant change in the titre of IgG antibodies to *C. trachomatis* but not to *N. gonorrhoeae*, *M. hominis*, or *U. urealyticum* was detected.

In those sections of the Fallopian tubes studied by transmission electron microscopy, no cellular inclusions consistent with those caused by chlamydia were noted. Ciliated cells could be seen but the cilia did not show any structural changes. The nucleus of the ciliated cells showed degenerative alterations with distorted morphology. The non-ciliated secretory

cells showed protrusions with plenty of microvilli indicating an increased luminal surface. In non-ciliated epithelial cells, an increased number of lysosomes suggesting an increased secretory activity was found.

The light microscopy findings of histological changes in the tubes from both patients were the same as those described in textbooks for gonococcal salpingitis (Novak and Woodruff, 1974). Furthermore, the inflammatory changes were similar to those in grivet monkeys experimentally infected in the tubes with *C. trachomatis* (Ripa *et al.*, 1979).

It seems that the old textbook differentiation of gonococcal and nongonococcal salpingitis ought to be re-evaluated.

This work was supported by Aarhus Universitets Forskningsfond 7131-01 and by the Swedish Medical Research Council grant 16X-4509.

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Prevalence of nine different micro-organisms in the female genital tract

A comparison between women from a venereal disease clinic and from a health control department

K. PERSSON,* KRISTINA PERSSON,† H. HANSSON,‡ B. BJERRE,‡
L. SVANBERG,‡ T. JOHNSSON,* AND A. FORSGREN†

From the Departments of *Clinical Virology, †Clinical Bacteriology, ‡Dermatovenereology and
‡Gynaecology, Malmö General Hospital, Malmö, Sweden

SUMMARY In a study of the prevalence of nine different micro-organisms in the female genital tract in a Swedish population, significantly higher isolation rates occurred among women attending a venereal disease clinic than among those attending a gynaecological health control department. The prevalence of *Candida albicans*, however, was similar in different groups, individual susceptibility being the most important factor. *Chlamydia trachomatis*, *Trichomonas vaginalis*, and *Mycoplasma hominis* occurred concomitantly with *Neisseria gonorrhoeae*, indicating a similar epidemiology for all these agents. Younger patients seemed to have an increased susceptibility to *C. trachomatis* whereas older patients had an increased susceptibility to *T. vaginalis*.

Introduction

Besides *Neisseria gonorrhoeae* genital symptoms can be caused by *Trichomonas vaginalis*, *Chlamydia trachomatis* (Dunlop *et al.*, 1972), *Mycoplasma hominis* (Mårdh *et al.*, 1975), *Candida albicans*, and herpes simplex virus. *Ureaplasma urealyticum* has not been connected with symptomatic infections in the female genital tract and its role in non-specific urethritis in men is still undecided, although recent findings have again indicated its role as a potential pathogen (Bowie *et al.*, 1977). Cytomegalovirus causes foetal damage and group B streptococci may cause neonatal infections, but neither has been associated with genital symptoms. We have investigated the occurrence and interrelationship of nine different micro-organisms found in the female genital tract. The isolation rates of a group of patients attending a department of venereal diseases were compared with those of a group of women attending the gynaecological health control department and with those of a group of young healthy girls seeking contraceptive advice.

Address for reprints: Dr K. Persson, Department of Clinical Virology, University of Lund, Malmö General Hospital, S-21401 Malmö, Sweden

Received for publication 7 February 1979

Patients and Methods

STUDY POPULATION

The present investigation included three groups of women.

Group 1

All untreated women were examined for the presence of *N. gonorrhoeae* and *C. trachomatis* at their first visit to the venereal disease clinic during 1977. The group consisted of 755 women, with a median age of 23 years and a range of 13-70 years. Three per cent of this group were immigrant women of European origin.

Additional cultures (390) were performed during the period February to June for *T. vaginalis*, *M. hominis*, *U. urealyticum*, group B streptococci, and *C. albicans*. Between August and October cultures were performed for herpes simplex virus and cytomegalovirus (319). Of the entire study population, 53 women were consorts of men who had positive culture results for both *N. gonorrhoeae* and *C. trachomatis*.

Group 2 (controls)

All women attending the gynaecological health control department, which covers over 90% of the

female population over 20 years of age, were examined for all nine micro-organisms during the period March to May. The group consisted of 201 women, with a median age of 26 years and a range of 23-30 years. About 13% were immigrant women of Finnish and Slavic origin, which is close to the average figure for the whole of Sweden.

Group 3 (controls)

A group of 111 young healthy girls seeking contraceptive advice at the department of gynaecology was examined for the presence of *N. gonorrhoeae*, *C. trachomatis*, herpes simplex virus (HSV), and cytomegalovirus during the period from September to October. The age of these girls ranged from 12 to 19 years, with a median age of 16 years. Two per cent of the group were immigrants.

ISOLATION PROCEDURE

Chlamydia

Specimens were obtained from the cervix with a cotton-wool swab, which was placed in modified 2 SP transport medium, in which 10% sorbitol had been substituted for sucrose (Richmond, 1974). Irradiated McCoy cell cultures were used and chlamydial inclusions were detected microscopically after being stained with iodine.

Herpes simplex virus and cytomegalovirus

The same specimen used for chlamydial isolation was also examined for herpes simplex virus and cytomegalovirus. Human embryonic lung fibroblast cultures were inoculated and positive isolates were identified as herpes simplex virus by a complement fixation (CF) test and as cytomegalovirus by direct immunofluorescence.

Group B streptococci

Cotton-wool swabs from the urethra and rectum

were placed in tubes containing a selective broth medium (Baker *et al.*, 1973). Suspicious organisms were identified as group B streptococci by serotyping (Christensen *et al.*, 1973).

Yeasts and T. vaginalis

Material was taken from the vaginal fornix, put into a tube with Diamond's broth (Diamond, 1957) and examined microscopically on days 3, 4, and 5.

Neisseria gonorrhoeae

Urethral, rectal, and cervical specimens were inoculated at the bedside on two chocolate-ascites agar plates, and positive isolates were confirmed by fermentation tests (Juhlin, 1963).

Mycoplasma hominis and Ureaplasma urealyticum

Modified Shepard agar and a broth of modified Shepard medium with urea were inoculated at the bedside. The diagnosis of *M. hominis* was made microscopically, and *U. urealyticum* was identified by its ability to hydrolyse urea and by its microscopical appearance (Mårdh *et al.*, 1975).

All plates and tubes were immediately transported to the laboratories and were processed within four hours.

Statistical comparisons were made using the χ^2 test throughout.

Results

Details of the isolation rates for the different agents are shown in Table 1. Significantly higher rates were found in the study group (group 1) than in the control group (group 2) for all micro-organisms except *Candida*, for which the difference was not statistically significant. In control group 3 *N. gonorrhoeae* was found in one patient, *C. trachomatis* in nine, and cytomegalovirus (CMV) in

Table 1 Isolation rate for each of nine different micro-organisms

Micro-organism	Group 1 (study group)			Group 2 (control group)	
	No. of cultures	% isolation-positive for each agent	% of <i>N. gonorrhoeae</i> -positive cultures with positive isolation for other agents	No. of cultures	% Isolation-positive for each agent
<i>N. gonorrhoeae</i>	755	28		200	0.5
<i>C. trachomatis</i>	755	25	40	201	5
<i>M. hominis</i>	382	41	53	200	19
<i>T. vaginalis</i>	390	12	20	201	1
<i>U. urealyticum</i>	382	75	74	200	60
Group B streptococci	379	41	40	189	28
Cytomegalovirus	319	7	9	201	1
Herpes simplex virus	319	4	7	201	0.5
Yeasts	390	25	19	201	22

Statistical difference between study group and control group for *N. gonorrhoeae*, *C. trachomatis*, *M. hominis*, *T. vaginalis*, and *U. urealyticum*, $P < 0.001$; for group B streptococci, CMV, and HSV, $P < 0.01$; and for yeasts, $P > 0.05$.

four, which is a significantly lower rate than in group 1. Associations between *N. gonorrhoeae* and *C. trachomatis* ($P<0.001$), *T. vaginalis* ($P<0.01$), and *M. hominis* ($P<0.01$) were evident in group 1 and could not be analysed in the control groups, as the number of positive isolates was too small.

CONCOMITANT INFECTIONS

In the study group (group 1) 40% of patients harbouring *N. gonorrhoeae* also harboured *C. trachomatis* compared with 20% of the patients without gonorrhoea; similarly, 53% harboured *M. hominis* and 20% *T. vaginalis* of those patients who also harboured *N. gonorrhoeae* compared with 37% and 9% respectively of those patients without gonorrhoea (Table 1). No correlation was found between the isolation of *N. gonorrhoeae* and that of group B streptococci, *U. urealyticum*, HSV, CMV, or yeasts.

Of the nine different micro-organisms studied, on average three or four were detected among those patients in group 1 who harboured *N. gonorrhoeae* (Fig. 1). The mean number of isolated micro-organisms was highest for patients who harboured *N. gonorrhoeae*. A lower mean number was found for those patients who harboured *C. trachomatis*, and the lowest mean number occurred among those with concomitant infection with *U. urealyticum*.

AGE FACTOR

The prevalence of *N. gonorrhoeae* and *C. trachomatis* in the study group (group 1) was the same, with a peak around 20 years (Fig. 2). The prevalence of *N. gonorrhoeae* and *C. trachomatis* for the different ages in this group are also shown in Table 2. The isolation rate for *N. gonorrhoeae* in the six age groups did not show a significant difference between the groups by the χ^2 test. The rate for *C. trachomatis* for the same age groups, however,

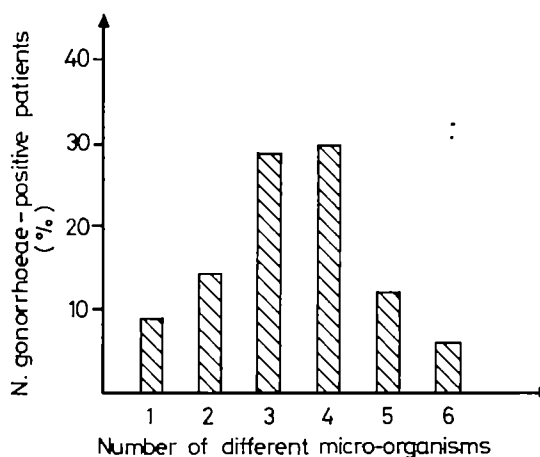


Fig. 1 Percentage of patients harbouring *N. gonorrhoeae* in the study group in relation to the number of agents isolated

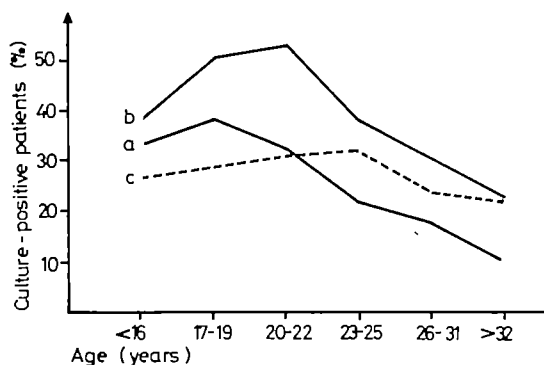


Fig. 2 Distribution of *N. gonorrhoeae* and *C. trachomatis* for different age ranges of the study group (group 1): (a) — *C. trachomatis* in group 1; (b) — *C. trachomatis* in patients with gonorrhoea (group 1); (c) - - *N. gonorrhoeae* in group 1

Table 2 Isolation rate for three different micro-organisms compared with that for *N. gonorrhoeae* in different age ranges for study group (group 1) and two further groups of patients attending the venereal disease clinic (groups 1a and 1b)

Age groups	Group 1			Group 1a			Group 1b		
	No. of patients	Positive isolation results (%) for		No. of patients	Positive isolation results (%) for		No. of patients	Positive isolation results (%) for	
		<i>C. trachomatis</i> *	<i>N. gonorrhoeae</i> †		<i>M. hominis</i> ‡	<i>N. gonorrhoeae</i> §		<i>T. vaginalis</i>	<i>N. gonorrhoeae</i>
Under 16	48	33	27	34	41	21	34	3	21
17-19	161	38	29	69	38	26	73	3	26
20-22	139	32	27	71	30	25	72	11	29
23-25	146	22	32	77	44	32	79	18	34
26-31	139	18	24	64	47	17	62	15	16
32 and over	122	10	22	61	48	31	64	19	28
Total	755	25	28	376	41	26	384	12	27

Statistical differences: *C. trachomatis*, $\chi^2 = 28.81$, $P<0.001$; *N. gonorrhoeae*, $\chi^2 = 3.23$ (group 1), $\chi^2 = 4.16$ (group 1a), $\chi^2 = 4.97$ (group 1b); *M. hominis*, $\chi^2 = 3.81$; *T. vaginalis*, $\chi^2 = 12.54$, $P<0.05$

exceeded chance variation between the groups ($P < 0.001$).

The same statistical comparison was made for 376 patients (group 1a) for whom isolation results were available for both *M. hominis* and *N. gonorrhoeae* (Table 2). The age groups did not differ significantly from each other for the isolation of either *M. hominis* or *N. gonorrhoeae*.

A group of 384 patients (group 1b) with isolation results for both *T. vaginalis* and *N. gonorrhoeae* was also analysed (Table 2). The isolation rate for *T. vaginalis* differed in different age groups to a statistically significant degree ($P < 0.05$). The rates for *N. gonorrhoeae* were within the limits of normal variation. Thus, there was a higher likelihood of young patients harbouring *C. trachomatis* than older ones and a higher likelihood of *T. vaginalis* occurring in the older age groups. The isolation of *N. gonorrhoeae* and *M. hominis* was evenly distributed and did not occur more frequently in any particular age group.

Of the 53 female consorts of men who harboured both *N. gonorrhoeae* and *C. trachomatis*, 43 (81%) harboured *N. gonorrhoeae* and 23 (50%) harboured *C. trachomatis*. Twenty consorts harboured both agents. Thus, the isolation rate for *C. trachomatis* was significantly lower than that for *N. gonorrhoeae* ($P < 0.001$) in these patients.

Discussion

In this study all agents except yeasts were found significantly more often among patients attending the venereal disease clinic (group 1) than among those attending the health control department (group 2). *C. trachomatis*, *T. vaginalis*, and *M. hominis* were strongly associated with *N. gonorrhoeae*, which would suggest that they have a similar epidemiology and are sexually transmitted. Reactivation of a latent chlamydial infection has been suggested by Richmond and Sparling (1976), which could also be manifested as a statistical correlation with *N. gonorrhoeae*. The study group patients harbouring *N. gonorrhoeae* had the highest mean number of isolated micro-organisms, which could indicate that these patients were the most heavily exposed to all of the six agents.

A higher prevalence of *U. urealyticum* and group B streptococci in the study group than in the healthy control group might occur for different reasons. Sexual transmission cannot be ruled out but would seem to be of minor importance, since the high prevalence of these organisms in the study group might be due to the selection of patients in this group. These saprophytic organisms seem to be

carried in the female genital tract. No association between group B streptococci and *N. gonorrhoeae* was found, which agrees with earlier reports (Wallin and Forsgren, 1975).

CMV was found more frequently in the study group than in the other groups. Sexual transmission of CMV has been suggested (Jordan *et al.*, 1973) and as virus secretion might continue for months and even years a higher rate of isolation in the study group would be expected. HSV was also found more often in the study group. Sexual transmission is a likely cause of primary infection (Beilby *et al.*, 1968). Recurrence of HSV infection is common and may be initiated by another genital infection. The occurrence of HSV infection is probably an effect of both sexual transmission and reactivation of a latent infection possibly stimulated by some concomitant infectious agent.

Yeasts did not occur significantly more often in either the study group or the healthy control group. Comparable results have been reported by others (Hilton *et al.*, 1974). The occurrence of yeasts was not related to the epidemiology of venereal disease agents such as *N. gonorrhoeae* and *C. trachomatis*. Colonisation by yeasts thus seemed to depend to a great extent on the individual susceptibility of the patients, in whom local pH values, hormonal factors, and microbial competition might play a part.

The age distribution of *C. trachomatis* and *T. vaginalis* was uneven. If these agents circulated freely in the different age ranges the results would indicate an increased susceptibility for *C. trachomatis* in younger women, who might perhaps develop an immunity later, and an increased susceptibility to *T. vaginalis* in the older age group.

C. trachomatis was found significantly less frequently than *N. gonorrhoeae* among female consorts of men who harboured both agents. The isolation techniques for these two micro-organisms are unlikely to be equally sensitive, which partly accounts for the difference in prevalence. Other factors such as differences in infectivity and susceptibility might also have influenced the results.

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Effect of hysterectomy on genital infections

FRANKLYN N. JUDSON AND MICHAEL A. RUDER

From the Denver Metro Health Clinic, Disease Control Service, Denver Department of Health and Hospitals, Denver, Colorado, USA

SUMMARY In a study of women with anogenital gonorrhoea who had undergone hysterectomy, the sensitivities of urethral, vaginal, and anal canal cultures were 88.9, 55.7, and 40.7% respectively in specimens from 27 women. To obtain the greatest sensitivity, however, we recommend that specimens from all three sites should be cultured routinely. After the raising effects of trichomoniasis and menstrual blood on the pH values have been allowed for, the vaginal pH of 74 women in the study group and of 137 healthy controls was similar.

Introduction

The prevalence and course of sexually transmitted genital infections could theoretically be influenced by the absence of the uterine body and cervix. Several factors may play a part, such as the predilection of gonococci for endocervical columnar epithelium (Harkness, 1948), the pH-buffering effect of cervical mucus (Peeters *et al.*, 1972), the bacteriological effects of mucin (Enhoring *et al.*, 1970), the competition from endocervical bacterial flora and their metabolic products (Bartlett *et al.*, 1978), and the presence of menstrual blood (Cohen, 1969). Our aim was to study how surgical removal of the cervix affects the prevalence of sexually transmitted genital infections by comparing disease-specific infection rates in women who had undergone hysterectomy with rates in a control group of women with an intact uterus. In addition, we have attempted to identify the most sensitive site for culturing *Neisseria gonorrhoeae* from women after hysterectomy.

Patients and methods

The Denver Metro Health Clinic is a major clinic for the treatment of sexually transmitted diseases (STD) in the Denver metropolitan area. The clinic records about 22 000 patient visits each year and more than 65% of the cases of gonorrhoea reported from Denver are treated there.

A standardised clinic visit form was used to collect an extensive STD data base for each patient

(Rothenberg and Judson, 1979). The data base did not include the reason(s) for the hysterectomy or whether or not oophorectomy had also been performed. In this study, women were considered to be 'symptomatic' if they complained of vaginal discharge.

CULTURE TECHNIQUES

At the initial visit (a first-time or a subsequent visit more than 30 days after a previous visit), two specimens for culture for gonococci were obtained from the endocervix and one from the anal canal (without a proctoscope) of healthy control women whereas one specimen was obtained from each of the urethra, posterior vaginal fornix, and anal canal of women who had undergone hysterectomy. Calcium-alginate urethrogenital swabs were used for urethral specimens whereas cotton-tipped swabs were used for specimens from other sites. Specimens were streaked directly on to modified Thayer-Martin medium and placed in an incubator at 36°C in an atmosphere containing 5-10% carbon dioxide. After 40-48 hours' incubation, cultures showing morphologically typical oxidase-positive colonies of Gram-negative diplococci were considered to give a positive result.

Cotton-swab specimens for saline wet preparations and 10% potassium hydroxide (KOH) mounts were obtained from the posterior vaginal fornix of all women and examined microscopically within five minutes for the presence of trichomonads and yeasts. Vaginal discharge was graded according to (a) quantity—none, scanty (insufficient quantity to flow into the lower half of the speculum), moderate (sufficient quantity to flow into the speculum but not from the introitus), and profuse (spontaneous flow from the introitus); and (b) quality—clear, white, or

Address for reprints: Dr F. N. Judson, Disease Control Service, 605 Bannock Street, Denver, Colorado 80204, USA

Received for publication 5 March 1979

purulent. Finally, the pH value of the posterior vaginal secretions was determined with nitrazine paper (phenolphthiazine paper, E. R. Squibb and Sons Inc., Princeton, New Jersey) in all study group women and in a 50% sample of the healthy control group.

DEMOGRAPHIC DATA

Study group

From 26 May 1977 to 13 May 1978 91 women under 40 years of age who had undergone hysterectomy presented to the clinic. The age limit was applied to avoid any effects of the menopause on the vaginal pH value and on the infection rates of the control group of women.

Control group

Out of 8141 women with an intact uterus seen during the same period a control group of 264 women (three controls for 82 women and two controls for nine women), under 40 years of age was selected by computer for identical composition to the study group for age (mean 32.6 years), race (78% white, 10% black, 10% Hispanic, and 2% others), sexual activity (mean 2.1 different sexual partners within 30 days of the clinic visit), period since most recent sexual contact (mean 5.8 days), and history of exposure to gonorrhoea (15.4% had been exposed within 30 days of the clinic visit).

To determine the relative sensitivities of urethral, vaginal, and anal canal cultures for *N. gonorrhoeae* more accurately, we continued to investigate the study group until December 1978.

Differences between the study group and the control group were analysed using a critical ratio for the difference of proportions (z test). The differences in pH were examined with an unpaired Student's *t* test for comparison of means.

Results

Vaginal discharge was significantly ($z=3.5847$, $P<0.001$) more common in the study group (77%) than in the control group (56%). This difference was also noted for subgroups with gonorrhoea (75%

compared with 44%), trichomoniasis (88% compared with 76%) and yeast infection (90% compared with 74%). In contrast, there was no significant difference between the two groups in terms of quantity and quality of vaginal discharge as determined by the clinician during vaginal examination. Thus, it appears that women who have undergone hysterectomy are more aware, or less tolerant, of vaginal discharge.

Comparative rates of genital infection are shown in Table 1. Trichomoniasis was significantly more common in the study group than in the controls; on the other hand, for gonorrhoea and yeast infection there were no significant differences. In the study group, seven (50.6%) of 14 women exposed to gonorrhoea were infected compared with 19 (65.5%) of 29 in the control group (P =not significant).

In Table 2, the range of posterior vaginal pH

Table 2 Comparison of vaginal pH in 74 women who had undergone hysterectomy and in 137 healthy controls, none of whom had trichomoniasis or were menstruating

pH value	Study group (n=74)		Control group (n=137)	
	No.	% of total	No.	% of total
4.5	34	45.9	54	41.9
5.0	14	18.9	28	21.7
5.5	11	14.9	19	14.7
6.0	8	10.8	20	15.5
6.5	5	6.8	7	5.4
7.0	2	2.7	8	6.2
7.5	0	0	1	0.8

values in 74 women in the study group are compared with that in 137 women in the control group who did not have trichomoniasis and who were not menstruating (controls only). The mean pH value of women in the study group was 5.1, which was similar to the mean of 5.2 for the healthy controls. The mean pH of women with trichomoniasis in both groups (5.6 and 5.8) was higher than that for women with no infection and no abnormal discharge (4.8 and 5.1) (Table 3).

Of 27 women in the study group who had *N. gonorrhoeae* cultured from any site (Table 4), the urethra gave a positive result in 88.9% and was the

Table 1 Rates of gonorrhoea, trichomoniasis, and yeast vaginal infection in 91 women after hysterectomy and in 264 healthy controls

Infection	Study group (n=91)		Control group (n=264)		z test*	Probability
	No.	% of total	No.	% of total		
Gonorrhoea	12	13.2	34	12.9	0.0755	NS
Trichomoniasis	17	18.7	23	8.7	2.5937	<0.01
Yeast	10	11.0	19	7.2	1.1389	NS

*Critical ratio for the difference of proportions
NS = not significant

Table 3 Comparison of vaginal pH in 61 women after hysterectomy, and in 99 healthy controls, some of whom had gonorrhoea, trichomoniasis, yeast infection, or no genital infection or abnormal discharge

Infection	Study group (n = 61)		Control group (n = 99)	
	No.	Mean pH	No.	Mean pH
Gonorrhoea	12	5.2	20	5.5
Trichomoniasis	17	5.6	11	5.8
Yeast	10	4.8	12	5.2
No infection and no abnormal discharge	22	4.8	55	5.1

Table 4 Comparison of urethral and vaginal culture results in 27 women with anogenital gonorrhoea who had undergone hysterectomy

Culture results for specimens from			
Urethra	Vagina	No.	% of total
+	+	13	48.2
+	-	11	40.7
-	+	2	7.4
-	-	1*	3.7

+ Positive - negative

* Result from anal canal alone was positive

only site giving a positive result in 40.1% compared with the vagina, which gave a positive result in 55.6% and was the only site giving a positive result in 7.4%. The anal canal gave positive results in 11 (40.7%) and was the only site giving a positive result in one (3.7%).

Discussion

The uterine cervix is a secretory organ lined by mucus-producing columnar epithelial cells which serves as a conduit for endometrial secretions in one direction and spermatozoa and microbes in the other. Once genital contact with a pathogen has occurred, the cervix can affect both the rate of acquisition and the course of sexually transmitted genital infections.

Because *N. gonorrhoeae* is more frequently isolated from the endocervix than from the vagina (Dans and Judson, 1975), it has been argued that the endocervix is preferentially susceptible to gonorrhoea, possibly because of its columnar epithelial lining (Harkness, 1948). Brown and Lucas (1973) have even stated that 'the gonococcus is unable to penetrate normal stratified squamous epithelium and infection develops only on those surfaces lined with vulnerable columnar epithelium'. However, this opinion is contradicted by Evans's (1977) ultrastructural studies, which show primary squamous cell infection, and by clinical studies that document infections of the posterior pharynx

(Wiesner *et al.*, 1973), the vagina and not the cervix in prepubescent girls (Harkness, 1948), the vagina in women after hysterectomy (Klaus *et al.*, 1978), the urethra in women (Dans *et al.*, 1975), and the anal canal in men and women (Dans *et al.*, 1975).

Harkness (1948) has proposed that while the gonococcus may infect vaginal squamous epithelium it more avidly attacks columnar epithelium and hence the cervix with its abundance of this cell type. Evans (1977), however, was unable to demonstrate gonococci in or on cervical epithelium from 25 women with gonococcal cervicitis, finding it instead within and on stratified squamous epithelium of the portio vaginalis. Thus, the question of which cell type *N. gonorrhoeae* prefers remains unanswered.

Other factors which might be important to preferential endocervical survival of gonococci may be the qualitatively different microflora (Bartlett *et al.*, 1978) and the higher pH of the cervical os. The usual pH value of secretions from the os is about 6.8 (Peeters *et al.*, 1972) compared with 5.0 for vaginal secretions and 7.5 for the optimal growth of *N. gonorrhoeae* (Cohen, 1969). Cervical mucus itself may cyclically affect bacterial growth since it has been shown to be bactericidal for *Proteus mirabilis* (Enhoring *et al.*, 1970). A recent study by Bartlett *et al.* (1978) comparing cervical and vaginal bacterial flora undermines much of this argument, for on the whole there were no quantitative differences in the flora of the two sites.

Furthermore, menstrual blood which passes through the uterine cervix is an excellent culture medium and could buffer vaginal pH into a range more conducive to the growth of *N. gonorrhoeae*. In reality this may not be important, as Dans and Judson (1975) have shown that endocervical culture positivity rates do not vary during the menstrual cycle.

Hysterectomy, by removing any predisposing effects of the endocervix to gonococci and by leaving behind primarily vaginal and urethral stratified squamous epithelium, should provide a model to resolve partly some of these controversies. We found no significant difference between the prevalence rates of genital gonorrhoea in women who had undergone hysterectomy and in matched healthy women. More importantly, the gonorrhoea attack rate in women exposed to gonorrhoea after hysterectomy was similar to that in healthy women exposed to gonorrhoea ($P > 0.2$). Thus, although the gonococcus has been assumed to favour cervical epithelium, it establishes infection quite well in its absence. Unfortunately, our study methods did not permit us to determine what, if any, contribution was made by infection of columnar epithelium within perivaginal and periurethral glands to the positive culture rate.

For women with an intact uterus, the endocervix and anal canal have been the preferred sites from which to culture *N. gonorrhoeae* (Center for Disease Control, 1974). Similar guidelines for women who have undergone hysterectomy do not exist, but our study clearly indicates that urethral cultures are the most sensitive, detecting infection in 89% of 27 women with anogenital gonorrhoea after hysterectomy. This agrees, in general, with the recent report of Klaus *et al.* (1978), in which urethral, vaginal, and anal canal cultures gave a positive result in 100, 41, and 12% respectively of 17 women with anogenital gonorrhoea after hysterectomy.

Although we did not take urethral specimens for culture from our healthy women, previous studies have shown that after endocervical cultures vaginal rather than urethral cultures are the next most sensitive in detecting genital gonorrhoea. Thus, in 432 women with gonococci isolated at any anogenital site, Dans *et al.* (1975) found the endocervix to give a positive result in 96.1%, the vagina in 84.9%, and the urethra in 65%. Lucas *et al.* (1967) obtained similar results in 1268 women with gonorrhoea; the endocervix gave a positive result in 86%, the vagina in 79%, and the urethra in 60%. The greater sensitivity of a vaginal culture in women with an intact uterus is probably best explained by the discharging of viable gonococci from the cervix into the vagina.

As with the endocervix, the reasons for preferential colonisation of the urethra are not known but probably relate to the same, as yet poorly defined, combination of factors such as pH, competing microflora, and cell type. To obtain the greatest sensitivity, we recommend that specimens from all three sites be cultured routinely.

In Gry's study (1964) of the prevalence of *T. vaginalis* in 387 women, the organism was cultured from cervical specimens in only 13.1%. It was never cultured from the cervix alone, and thus the presence of a cervix seems to have little effect on the prevalence of trichomoniasis. However, optimal growth of *T. vaginalis* occurs during menses (Banner, 1974). If this were due solely to the buffering action of menstrual blood, we would expect a lower prevalence of trichomoniasis in women after hysterectomy. Inexplicably, we found trichomoniasis to be significantly more common in women who had undergone hysterectomy than in healthy women (18.7% compared with 8.7%, $P < 0.01$). This may result from our methods of selection in that a significantly greater proportion of women who had undergone hysterectomy complained of vaginal discharge. The explanation does not lie in vaginal acidity, because with or without trichomoniasis, the mean pH value of women after hysterectomy did not

significantly differ from that of the control women. It is not known whether the high pH associated with trichomoniasis is actually caused by the protozoon or instead helps to provide a favourable environment for infection (Cohen, 1969).

Candidosis is seldom sexually transmitted (Miles *et al.*, 1977) but is nonetheless a common genital infection which could be influenced by the presence of the cervix. Rosenberg (1976) found that only 44% of women with the 'typical symptoms complex' of candidosis had positive results when cultures were performed throughout the cycle, while 85% had positive results when cultures were performed within four days of the onset of menstruation. If a change in premenstrual cervical mucus was responsible, the prevalence of yeast infections in women after hysterectomy would be lower than in controls. However, we found yeasts on KOH preparations in 11% of women who had undergone hysterectomy and in 7.2% of control women ($P > 0.2$). As with trichomoniasis, the slightly higher prevalence of yeast infections in women after hysterectomy may be due to the higher percentage of these women who were symptomatic.

The automated clinic data system is supported by a contract from the Operations Research Branch, Venereal Disease Control Division, Center for Disease Control, Atlanta, Georgia. The contributions of Mr Arnold Maltz, in data programming, of Dr Richard Rothenberg, in statistical analysis, of Ms Fern Lemaster, for secretarial assistance, and of the staff of the Denver Metro Health Clinic are gratefully acknowledged.

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Rising incidence of chancroid in Rotterdam

Epidemiological, clinical, diagnostic, and therapeutic aspects

K. C. NAYYAR, E. STOLZ, AND M. F. MICHEL

From the Departments of Dermatovenereology and Clinical Microbiology, Erasmus University, Rotterdam, Holland

SUMMARY The incidence of chancroid in Rotterdam has increased by more than five-fold during 1977-78. In a retrospective study of 53 patients with chancroid seen at this clinic during this period, the results of smears were positive in 82% and of cultures in 84% (of those for whom cultures had been performed). Symptoms were generally mild. Treatment with co-trimoxazole was highly effective clinically, as confirmed by *in-vitro* sensitivity studies.

Introduction

Chancroid is usually considered to be a venereal disease which is prevalent mostly in tropical and sub-tropical countries. In the venereal disease clinic at University Hospital in Rotterdam, we previously used to diagnose chancroid in less than five patients a year until 1977. Using the same diagnostic techniques, we have observed a sudden increase in the incidence of chancroid in Rotterdam since 1977. For this reason, we have recorded retrospective data on 53 patients with chancroid (27 attending the clinic in 1977 and 26 in 1978).

Patients and methods

All records of patients with genital ulcers or inguinal bubos or both seen at this clinic in 1977 and 1978 were analysed. In addition to routine clinical examination, bacteriological investigations for the diagnosis of urethral gonorrhoea, using the method described by Stolz *et al.* (1974), and of trichomoniasis, using trichomonas medium RCLG (Ringer cysteine liver glucose containing penicillin G 1 megaunit/100 ml, streptomycin 1g/100 ml, and nystatin 50 000 u/100 ml) were carried out, as were serological tests and darkfield examination for syphilis. Samples were taken from the ulcers for culture for gonococci, trichomonads, and yeasts. Scrapings from the bases and the walls of the ulcers were smeared on slides and examined microscopically

for micro-organisms after being stained with Gram and Giemsa stains; cultures for herpes simplex virus and bacteria were performed for every patient.

Cultures for *Haemophilus ducreyi* were carried out using 25% fresh defibrinated rabbit blood in agar incubated for 48 hours in 10% CO₂ at 34°C. The organisms were identified by their typical morphology in Gram-stained and Giemsa-stained smears from suspect colonies, by their CO₂ requirement, and by the occurrence of haemolysis.

Co-trimoxazole sensitivity tests were carried out for 20 strains of *H. ducreyi* by the disc diffusion method using impregnated discs; these indicated a minimum inhibitory concentration (MIC) of 25 µg/ml with an inhibition zone of 28 mm.

During 1977 and 1978, 673 men and 23 women with genital ulcers were examined at our clinic. The diagnosis of chancroid, based on a positive smear result or positive culture result or both, was made in 50 (77%) men and in three (13%) women.

The first six patients were treated with kanamycin 1 g i.m. daily for five days. The following three patients were treated with tetracycline hydrochloride 2 g orally daily for 10 days. Thirty-one patients were treated with co-trimoxazole two tablets twice daily and 13 patients were treated with co-trimoxazole three tablets twice daily (each tablet containing 400 mg sulphamethoxazole and 80 mg trimethoprim) for 10-14 days consecutively.

Results

AGE AND NATIONALITY

The mean age of the 53 patients with chancroid was 30.4 years (range 19-53). The nationalities of the patients are given in Table 1.

Address for reprints: Dr K. C. Nayyar, Department of Dermatovenereology, Faculty of Medicine, Erasmus University, PO Box 1738, Rotterdam, Holland

Received for publication 28 March 1979

Table 1 *Nationalities of 53 patients with chancroid*

Nationality	No.	Nationality	No.
West European		Middle-Eastern	
Dutch	16	Turkish	6
Portuguese	8		
Spanish	3	African	
French	1	Moroccan	5
Belgian	1		
Danish	1	Asian	
British	1	Indian	1
German	1	Pakistani	1
Finnish	1	Ceylonese	1
Polish	1	Thai	1
Yugoslavian	1	Total	4
Total	35		
Unknown	2	South American	
		Chilean	1

OCCUPATION

Twenty-four patients were sailors, four factory workers, three businessmen, three prostitutes, and two civil servants; four were unemployed and in 13 the occupation was unknown.

COUNTRY OF ACQUISITION

The countries where the infection was probably acquired, based on the patient's history, are shown in Table 2. Prostitutes were the probable source of infection in 48 (89%) patients whereas friends or acquaintances infected five (11%) patients.

Table 2 *Probable country of acquisition of chancroid*

Country	No. of patients	Country	No. of patients
Europe		Africa	
Holland	15	Morocco	5
Portugal	7	Nigeria	3
Finland	1	South Africa	1
Belgium	1	Total	9
Spain	1		
Greece	1	Asia	
Total	26	India	2
		Bangladesh	2
South America		Nepal	1
Chile	1	Thailand	1
		Total	6
Middle East		Unknown	7
Turkey	4		

INCUBATION PERIOD

In 22 patients, for whom a definite history was available, the incubation period varied from two days to three weeks.

LESIONS

Ulcers occurred on the penis in 48 patients, in the groin and on the penis in one, in the perianal region in one, and on the vulva in three. Twenty-six patients had single penile lesions whereas 23 had multiple lesions. In 29 patients lymph nodes were enlarged. The lesions on the vulva occurred singly without

enlargement of the lymph nodes. In general, the symptoms were mild.

CULTURE AND MICROSCOPY

Results of Gram-stained and Giemsa-stained smears and cultures for the detection of *H. ducreyi* are given in Table 3. In all 20 strains of *H. ducreyi*, for which the sensitivity for co-trimoxazole was determined, a MIC < 25 µg/ml was found.

Table 3 *Results of Gram-stained and Giemsa-stained smears and cultures for H. ducreyi*

No. of patients	% of total	% of total*	Results	
			Smear	Culture
24	45	53	+	+
6	11	13	++	+
8	15	18	-	+
7	13	16	+	-
8	15		+	ND

+ Positive - negative

* Not including those patients for whom cultures were not performed

† Doubtful-positive

ND = not done

CHANCROID AND OTHER STDs

In addition to chancroid, two patients also had early latent syphilis, one primary syphilis and genital warts, one gonorrhoea, one trichomoniasis, one candidosis, one herpes simplex virus infection, and one gonorrhoea, trichomoniasis, and candidosis.

TREATMENT

The six patients treated with kanamycin were cured within one week whereas the three patients treated with tetracycline were cured within two weeks. Of the 44 patients treated with co-trimoxazole, 18 did not return for follow up. Among those who did return, there were no treatment failures and the time the ulcers took to heal varied from one to two weeks. None of the patients who attended after treatment complained of side effects.

Discussion

The finding of 53 cases of chancroid in this clinic in 1977 and in 1978, compared with less than five cases a year during the previous years, certainly shows a marked increase in its incidence in Rotterdam. In recent years a sudden increase has been reported by several workers in other countries. Morel (1974) observed an epidemic of chancroid in Paris during 1973. Lüders *et al.* (1975) reported a similar increase in West Germany. In the Netherlands, an increase in incidence has been reported by Baart de la Faille *et al.* (1977) and recently Murat *et al.* (1978) reported an

epidemic in Istanbul, Turkey. Most of our patients were either Dutch, Portuguese, Turkish, or Moroccan. Of the six Turks, four had been infected in Turkey. This finding may perhaps correlate with the recently reported epidemic in Turkey (Murat *et al.*, 1978). Of the Portuguese and Moroccans, only two were infected in Rotterdam whereas the remaining 11 had been infected in their own countries. Two Turks, two Portuguese, one Greek, one Spaniard, and nine Dutch, including the three prostitutes, had a history of sexual contact in the same part of Rotterdam, where prostitutes are frequently visited by immigrant workers. Before 1977, no patients with chancroid, infected either in Rotterdam or in Holland, were seen in our outpatient department. The possibility, therefore, that the infection is being imported from outside the Netherlands, must be considered. Prostitutes constitute a large reservoir of infection (Gaisin and Heaton, 1975); 89% of our patients acquired the infection from prostitutes, and all three women in our study were prostitutes.

In our study the smears of 82% of patients gave a positive result for *H. ducreyi*. This finding agrees with an earlier report by Reymen (1951), who also found that smears gave a positive result in 50-83% of cases. Had we only examined smears, we might have missed the diagnosis in eight (18%) patients. The fact that cultures gave negative results in 16% of patients with a positive smear result is more difficult to explain, but the reason may be that some smears gave a false-positive result and that the culture method was still not perfect.

H. ducreyi is resistant to sulphonamides (Marmer, 1972; Baart de la Faille *et al.*, 1977). Alternative drugs used by some workers are kanamycin (Marmer, 1972), gentamicin (Lüders *et al.*, 1975), and tetracycline (Murat *et al.*, 1978). Kanamycin and

gentamicin, however, are potentially toxic drugs and, moreover, need to be given parenterally. Tetracycline is treponemacidal and can therefore mask syphilis. Our studies showed that all the strains of *H. ducreyi* tested were sensitive to co-trimoxazole, and all patients responded well clinically.

Similar observations have also been reported by de Sablet and Puissant (1974) and by Morel (1974). In view of the disadvantages of kanamycin, gentamicin, and tetracycline and of our clinical experience with co-trimoxazole, the latter appears to be the drug of choice in the treatment of chancroid.

We gratefully acknowledge the technical assistance of Mrs G. E. Bosscher-Koetsier, Mrs M. P. L. de Jonge-Suy, Mrs S. de Weerd-van Ameyden, and Mr E. O'Niel.

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Effect of injections of small doses of human fibroblast interferon into genital warts

A pilot study

G. M. SCOTT AND G. W. CSONKA

From the Division of Communicable Diseases, Clinical Research Centre, Harrow, Middlesex, and the Praed Street Clinic, St Mary's Hospital, London

SUMMARY Eleven male patients with genital warts were given injections of fibroblast interferon (300 u) and placebo into the bases of two similar warts. The changes in size of the treated warts compared with that of the controls suggested that interferon did inhibit the growth of the warts. The dose given was probably insufficient for a dramatic effect. In one patient, however, a wart on the penile shaft was injected with interferon and did disappear within two weeks whereas an untreated meatal wart increased in size.

Introduction

Genital warts (condylomata accuminata) are a common sexually transmissible benign viral infection (Oriel and Almeida, 1970; Oriel, 1971). Treatment is usually with chemical or physical agents and is designed to remove the warty tissue. Infected cells tend to be left behind and the tumour commonly grows again. In a preliminary open study followed by a double-blind study comparing human leucocyte interferon ointment with placebo, interferon was effective in eliminating genital warts in most women treated (Ikić *et al.*, 1975a, b).

This study was designed to see if a single minimal dose of human fibroblast interferon (HFIF) injected into the base of a small genital wart would have any effect on its growth.

Patients and methods

Twelve male patients (age range 18-32) were admitted to the study. Details of the patients and their warts are shown in Table 1. All except one patient had warts on the penile shaft; the other had fleshy warts in the groin. All except one patient had two small warts of a similar size and appearance; one had a single wart on the shaft and a meatal wart (Patient 4).

Four patients had had warts for six months or longer and repeated applications of podophyllin had failed to remove them completely. Three others had had previous episodes of warts which had been

temporarily removed by podophyllin or electrocautery; the warts had recurred when treatment was stopped. Three patients experiencing their first attack had received no treatment whereas the other six had had podophyllin treatment which so far had not affected the warts. The most recent podophyllin application took place more than one month before treatment with interferon. Most patients had soft fleshy warts but one (Patient 7) had planar warts resembling verucca vulgaris.

The local ethical committee gave approval for the study; all patients were made conversant with the aims of the experiment and were assured that the treatment would not cure the warts. They then gave their written consent to the experiment.

After the injections the patients were instructed to attend weekly for three weeks. Only seven of the 12 patients complied exactly with the instructions but all except one were seen and assessed at the second or third week after interferon treatment.

PREPARATION OF INTERFERON

Solution A

The interferon solution used for the experiment was manufactured (G. D. Searle & Co. Ltd, High Wycombe) by induction of a newly derived foetal lung fibroblast cell line and superinduction using cycloheximide and actinomycin D according to the method of Sehgal *et al.* (1976). Interferon activity was measured by assessing the dilution which caused a 50% reduction in the cytopathic effect of Sindbis virus on Vero cells. Assay of the material at the end of the trial to break the code showed that solution A contained 3000 u/ml of interferon in two separate samples.

Address for reprints: Dr G. M. Scott, Division of Communicable Diseases, Clinical Research Centre, Harrow, Middlesex

Received for publication 30 November 1978

Table 1 Details of patients with genital warts treated with interferon (group A) or placebo (group B) or left as controls (group C)

Patient No.	Age	Previous episodes		Current episode		Success	Site	% change in wart size between first and last visit (1-3 weeks)		
		Presence of body warts	No.	Result of podophyllin treatment	Duration (months)	Other treatment		Group A	Group B	Group C
1	26	Yes	0		12	Podophyllin	Shaft	+49	-27	+90
2	31	No	1	Successful	3	None	Shaft	+20	0	-38
3	22	Yes	0		2	Podophyllin	Shaft + meatus	+35	+170	+165
4	19	No	0		6	None	Shaft + meatus	+100	+220	+220
5	32	No	Many	Intermittent success*	1	None	Shaft	0	+33	+73
6	27	Yes	0		6	Podophyllin	Shaft	+13	+44	+225
7	23	No	0		6	Podophyllin	Shaft	+8	+67	0
8	25	No	0		2	Podophyllin	Shaft	+46	+50	-7
9	19	No	0		3	None	Shaft	-17	-19	+37
10	18	Yes	0		2	Podophyllin	Shaft	-60	+26	0
11	30	Yes	0	(With cautery) intermittent success	4	None	Grain	-33	-34	+47
12	24	No	3		1½	None	Shaft	-56	+5	-32

* Complete clinical resolution of warts with subsequent recurrence

+ Failure to achieve complete resolution of disease

+ Plus - minus

Solution B

The control solution used was a mock fibroblast interferon derived from an abortive induction process and contained no antiviral activity in the above assay.

The cell-line interferon and mock interferon were subjected to stringent tests to ensure the absence of adventitious agents. Previous studies using intradermal injections of high titre fibroblast interferon (Scott *et al.* 1977, 1978) had shown no toxic effects except for painless local redness occurring four hours after injection and lasting for 24 hours.

INJECTION OF WARTS

Each volunteer received a single injection of 0.1 ml solution A (300 u interferon) into the base of one wart (group A warts) and 0.1 ml solution B (mock interferon) into another wart of similar size and appearance (group B warts). The materials were packed in single-dose syringes and the investigator was unaware which was the active and which the control solution. (Patient 4, who had one wart on the penile shaft and meatal wart, received an injection of solution A and the meatal wart was left as control.) All the warts not injected with either solution were left as controls (group C). All the warts were measured using dividers transferred to a mm scale, and the maximum and minimum diameters (mm) were recorded on the first day and then weekly for a maximum of three weeks.

REACTIONS

All patients reported that the injections caused intense local stinging for 5-10 seconds, but one patient volunteered that the stinging of the injections was less intense than the pain induced by electrocautery. There were mild erythematous reactions at the injection sites at 24 hours and no systemic symptoms.

Results

The area of the wart was calculated from the maximum (d max) and minimum (d min) diameters assuming they were elliptical;

$$\pi(d \text{ max}/2)(d \text{ min}/2)$$

Excluding patient 4, the mean size of warts was 10.0 mm² (range 2.4-20) in group A, 11.3 mm² (4.7-30) in group B, and 36.0 mm² (5.9-165) in the uninjected warts (group C). Changes in the size of warts are shown graphically in Figure 1. The proportional change in the size of the warts is shown in Figure 2.

STATISTICAL ANALYSIS

Two-way analysis of variance was performed on the

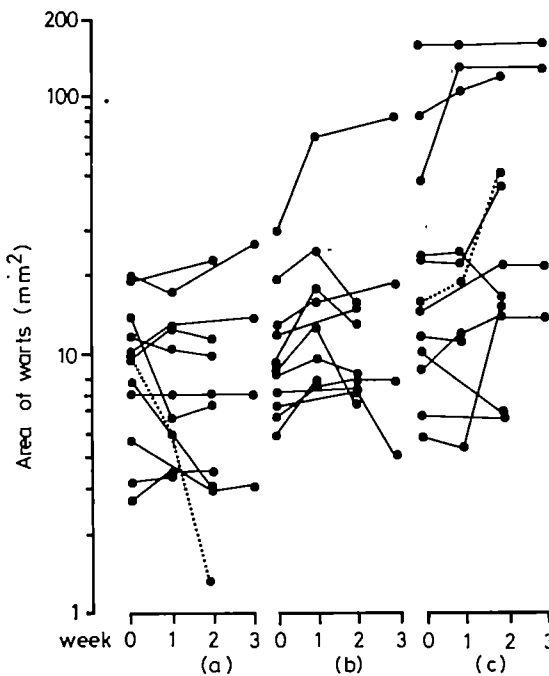


Fig. 1 Change in size of genital warts (a) treated with fibroblast interferon (group A); (b) treated with mock interferon (group B); and (c) untreated (group C) (.... Patient 4 did not receive interferon).

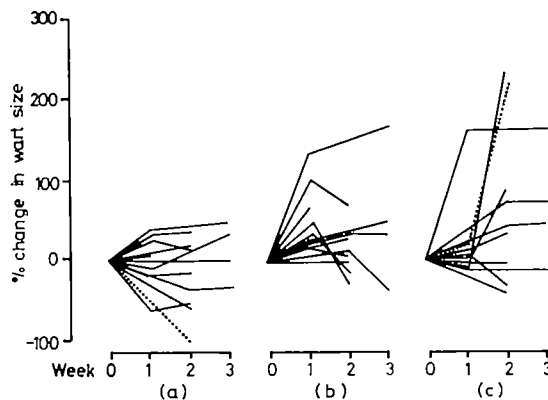


Fig. 2 Proportional change in size of genital warts (a) treated with interferon (group A); (b) treated with mock interferon (group B); and (c) untreated (group C). Excluding patient 4 (....) the difference between the mean geometric change in warts treated with interferon (-8%) and those treated with mock interferon (+19%) plus the untreated warts (+33%) is significant ($P=0.045$).

logarithm of the change in size of the warts between the first (pretreatment) and last (at one, two, or three weeks) attendance at the clinic before alternative treatment was instituted (Table 2). Patient 4, treated only with solution A, was excluded from the analysis. Patient 7 was assessed at one week; between the first and third weeks he was inadvertently treated with podophyllin.

The geometric mean of the ratio of the follow-up size to initial size was 0.92 (group A), 1.19 (group B), and 1.33 (group C). The overall difference between the three groups was not statistically significant. Between group A and groups B plus C, however, the difference was of borderline significance ($P=0.045$, two-tailed test). Thus warts treated with solution A (interferon) grew significantly less than all the other warts including those treated with mock interferon (solution B) and the untreated controls. There was no difference in the response to interferon in patients previously treated with podophyllin.

In the case of patient 4, a single wart on the penile shaft was treated with solution A (interferon) alone, and by the second week after injection the wart had disappeared leaving a faint scar. Meanwhile the untreated meatal wart had considerably increased in size.

Discussion

This pilot study indicates that the injection of a small dose of interferon may influence the growth of genital warts compared with that of controls. In one patient the wart injected with interferon disappeared within two weeks. Unfortunately there was no similar wart treated with mock interferon, so the disappearance could have occurred by chance, even though the untreated meatal wart increased in size.

Ikić *et al.* (1975a, b) studied the effect of relatively impure human leucoocyte interferon in an ointment base (4000 u/g) rubbed into vulval and vaginal warts five times a day. In an open study (Ikić *et al.*, 1975a), 36 of 40 women had complete resolution of their warts by the eighth week of treatment. In a double-blind study of 20 women with warts (Ikić *et al.*, 1975b), those in the interferon-treated group regressed more frequently than in the placebo group. Regression of the wart took as long as 12 weeks in the treated group but more usually four to five weeks.

The results of this study are encouraging considering the low activity of the interferon solution used. Repeated injections will not be tolerated by these patients and a double-blind comparison of a topically applied preparation of fibroblast interferon with an identical placebo is proposed when material is available.

Table 2 Analysis of variance on $\log_{10}$ (size of warts after treatment/initial size) in groups A (treated with interferon), B (treated with control), and C (untreated warts)

Source of variation	Sum of squares	d.f.	Mean square	Mean square ratio	Significance
Between groups	0.144624	2	0.072312	2.508	NS ($P > 0.1$)
A vs B	(0.066550)	(1)	0.066550	2.308	NS ($P > 0.1$)
B vs C	(0.012768)	(1)	0.012768	0.443	NS ($P > 0.1$)
A vs B, C	(0.131856)	(1)	0.131856	4.573	$P = 0.045$
Between patients	0.656321	10	0.065632	2.276	$P = 0.056$
Error	0.576643	20	0.028832		
Total	1.377588	32			

d.f. = degrees of freedom

NS = not significant

We are grateful to Mr J. R. Shelton for his statistical advice and to the staff of the Special Clinic, Central Middlesex Hospital, for their help.

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Perigenital cutaneous schistosomiasis

F. A. B. ADEYEMI-DORO,* A. O. OSOBA,* AND T. A. JUNAID†

From the *Special Treatment Clinic and the Venereal Diseases and Treponematoses Research Laboratories; and the †Department of Pathology, University College Hospital, Ibadan, Nigeria

SUMMARY Perigenital cutaneous schistosomiasis was diagnosed in a patient who had no previous genitourinary or gastrointestinal symptoms suggesting schistosomiasis; his only symptom was a pruritic papular rash in the perineum. Late cutaneous schistosomiasis due to deposition of ova in the dermis is rare but can affect the genital and periumbilical areas. This report highlights the difficulty in diagnosing cutaneous schistosomiasis and the need for biopsy of itchy cutaneous lesions in patients from localities where the infection is endemic.

Introduction

Schistosomiasis is a widespread disease with variation in the predominant species of causative organism in different areas. In Nigeria *Schistosoma haematobium* and *Schistosoma mansoni* are responsible for most cases, causing serious vesical and gastrointestinal lesions respectively (Edington and Gilles, 1971).

The cutaneous manifestations of schistosomiasis result from: (a) penetration of the skin by the infective stage cercariae resulting in schistosomal dermatitis; (b) urticaria, oedema, fever, and pruritus which are hypersensitivity manifestations of invasion; and (c) deposition of ova in the dermis and subcutaneous tissues by adult worms which have passed into the superficial veins, usually becoming manifest several months after systemic invasion (MacDonald and Morrison, 1976). Although cutaneous ectopic schistosomiasis around the umbilicus has been described, deposition of ova in the genital area is very rare. We decided to report this case of perigenital cutaneous schistosomiasis, at the same time focussing attention on the genital manifestations of this tropical disease.

Case report

A 10-year-old Nigerian boy was referred to the Special Treatment Clinic for the investigation of a three-month history of perineal rash and pruritus,

which had persisted despite the application of various creams with and without antimicrobial agents. The onset of the eruption could not be attributed to any factor. He had no constitutional symptoms and no history of cough or episodes of haematuria and had had no gastrointestinal symptoms in recent months. He had never had sexual exposure and there was no history of urethral discharge or genital ulceration.

On examination numerous, discrete, firm, painless, papular eruptions were visible in the perineum (Fig. 1). No other physical sign was found and the patient was given a topical antipruritic agent while awaiting the result of our investigations.

INVESTIGATIONS

Skin biopsy showed both viable and calcified schistosome ova within the epidermis and in the dermis (Fig. 2). Tissue reaction around these varied from predominantly eosinophilic polymorphonuclear infiltrates to a granulomatous picture with few giant cells. The schistosome ova showed a terminal spine characteristic of *S. haematobium* (Fig. 3). Urine examination on four occasions showed no red or white blood cells. No schistosome ova were present and no pathogens were cultured.

The white cell count was within the normal range with a relative eosinophilia while the schistosome complement fixation test gave a positive result. Heat test gave a grade I positive result and no mycobacterium was isolated from the biopsy material. Tissue culture for viruses also gave a negative result. Stool examination showed no schistosome ova. The Venereal Disease Research Laboratory test gave a negative result. Chest and abdominal radiographs and intravenous urogram were normal.

Address for reprints: Dr A. O. Osoba, Department of Medical Microbiology, University College Hospital, Ibadan, Nigeria

Received for publication 18 April 1979



Fig. 1 Discrete, firm, painless, papular eruptions in the perineum

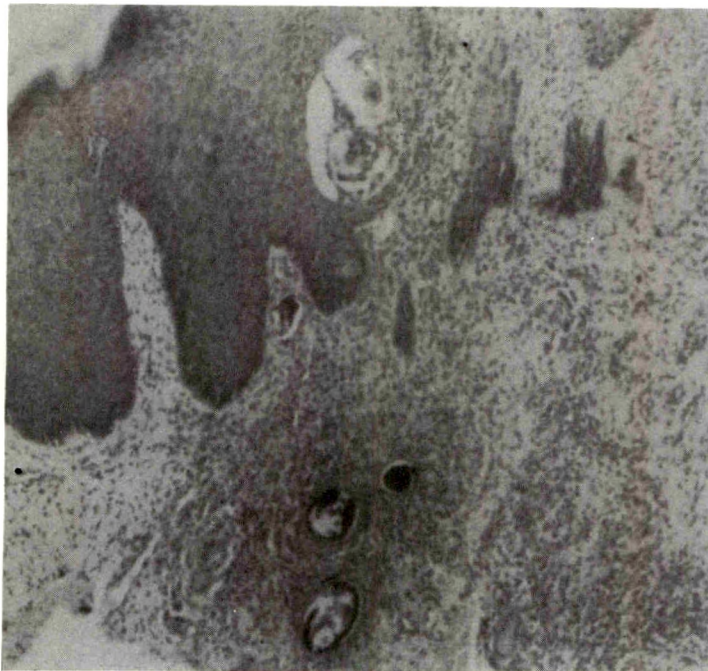


Fig. 2 Clusters of schistosome ova in dermis associated with chronic inflammatory cells including eosinophils (Haematoxylin and eosin $\times 100$)

The patient was treated with two courses of niridazole (Ambilhar) 100 mg thrice daily for a week; a good response was achieved within six weeks of treatment with remission of the pruritus and disappearance of the eruption.

Discussion

Schistosomiasis is endemic in many areas of the world. In Ibadan, Nigeria, *S. haematobium* is widely endemic (Edington *et al.*, 1970). Man is infected by

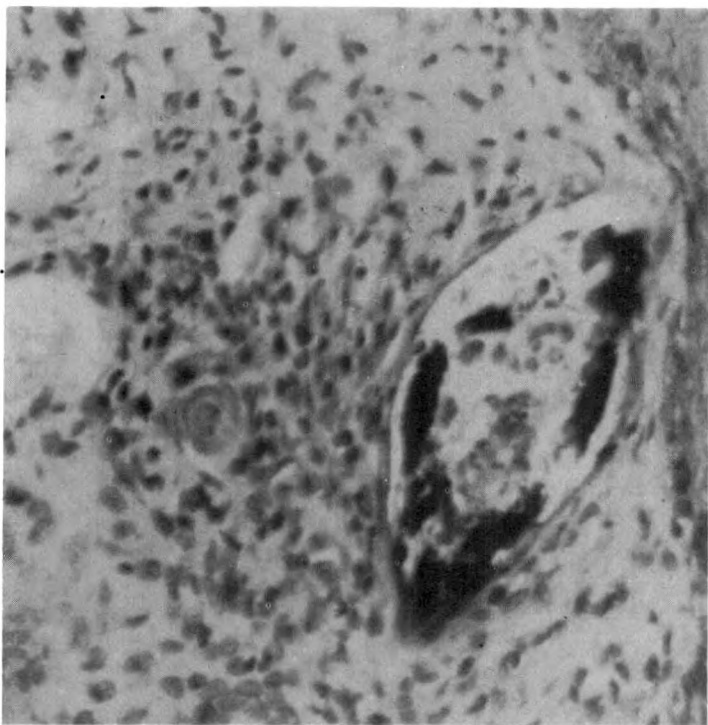


Fig. 3 Ova with terminal spine characteristic of *S. haematobium* amidst inflammatory infiltrate in the dermis (Haematoxylin and eosin $\times 190$)

active penetration of the intact skin by cercariae which have emerged from the fresh-water snail intermediate host. The cercariae migrate to various tissues of the body and finally enter the systemic blood stream by way of the lymphatics; they mature into adult worms in the intrahepatic vessels. When sexually mature, and depending on the species, the adult worm then migrates to the mesenteric veins (*S. mansoni*, *Schistosoma japonicum*, *Schistosoma intercolatum*) or to veins of the pelvic plexuses (*S. haematobium*) where natural egg production begins. The period between infection—that is, cercarial penetration of the skin and recovery of eggs from the stool or urine of the host—is usually four to six weeks (Woodruff, 1974). Black (1945) found a correlation between the time of appearance of eggs in the urine and the onset of cutaneous lesions. The mechanism responsible for their selective organ habitation is not fully understood.

Renal or gastrointestinal symptoms, for which treatment is usually sought several months earlier, almost invariably antedate the onset of cutaneous schistosomiasis. Even in areas where the disease is endemic the cutaneous lesions due to deposited eggs are rarely seen (Findley and Whiting, 1971; MacDonald and Morrison, 1976). The lesions are small, deep-seated, and discrete with numerous deposits in the subcutaneous tissue during early

stages (Black, 1945). The early lesion therefore resembles subcutaneous tuberculosis and should be differentiated from it by biopsy of the lesion, which shows a granulomatous infiltration with a preponderance of eosinophils in which viable or calcified schistosome ova or both are found in groups—so called 'schistosomal tubercles'. Tissue eosinophilia is usually associated with peripheral blood eosinophilia, as shown in this case.

Cutaneous schistosomiasis is believed to arise from deposition of ova by adult female worms that have migrated to the superficial veins through anastomoses between the superficial veins and the pelvic venous plexuses. The production of a perigenital cutaneous lesion, as in this case, might have been due to adult worms that strayed from their normal path into the perineal vessels. Inadequate treatment of previous infection (Haber and Cowley, 1957) and altered host-parasite relationship may be contributory factors in the development of ectopic cutaneous lesions (Findley and Whiting, 1971).

We have no explanation for the absence of previous renal or gastrointestinal symptoms in our patient, but gastroenteritis is so common in our locality that it may have been overlooked. Identification of the species of schistosome in skin sections can be made by serial sections of the biopsy material for the position of the spine of the eggs,

which is lateral in *S. mansoni* and terminal in *S. haematobium*. In our case the position was terminal indicating that the species was *S. haematobium*.

Successful treatment of schistosomiasis with niridazole (Ambilhar) has been previously reported (Mahmoud and Warren, 1974; MacDonald and Morrison, 1976).

This case illustrates the difficulties in diagnosing genital lesions that may be encountered in tropical countries, since tropical diseases may affect the genital areas and thus need to be considered in the differential diagnosis of unusual lesions of the genital area.

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Correspondence

TO THE EDITOR, *British Journal of Venereal Diseases*

Asymptomatic gonorrhoea among patients with condylomata acuminata

Sir,

There have been several studies reporting on asymptomatic gonorrhoea in both women and men (Carpenter and Westphal, 1940; Jones and Price, 1957; Lucas *et al.*, 1967; Pedersen and Harrah, 1970; Pariser, 1972; Handsfield *et al.*, 1974) and the incidence differs depending on the group studied. Those groups with the greatest chance of exposure to sexually transmitted diseases have the highest incidence of asymptomatic gonorrhoea (Thatcher *et al.*, 1969).

The present study was undertaken to evaluate the frequency of gonococcal infection in asymptomatic men with condylomata acuminata.

Sixty-seven male patients with condylomata acuminata who had no signs or symptoms (dysuria and urethral discharge) of gonococcal infection were included in the study. All patients admitted having sexual contact with prostitutes. Their ages ranged from 21 to 48 years (average 22 years); 84% were unmarried.

The following examinations were done in sequence: the entire urethra was compressed, and only those patients without demonstrable exudate were included in the study. Smear and culture examination of the anterior urethra were performed. A platinum loop (at least 5 cm long) was passed 3-4 cm into the urethra and with-

drawn along the mucosa. This urethral scraping was then used to prepare smears for Gram staining. A second loop was used to inoculate Thayer-Martin agar.

Gram-stained smears were considered to give a positive result if typical Gram-negative diplococci were seen, either intracellular or extracellular. Colonies isolated on Thayer-Martin were identified by Gram stain, oxidase test, and sugar fermentation reactions.

Of the 67 asymptomatic men with condylomata acuminata *Neisseria gonorrhoeae* was cultured from the anterior urethra in 23 (34%). Of these 23 cases, six gave a positive result by culture alone. No patient with a negative urethral culture result had a positive result to Gram stain.

Of the 67 men, five (7.1%) admitted having had gonorrhoea in the past, on average three months previously. All had been treated and their symptoms disappeared, although none had had follow-up cultures performed. Two others said they had had transient dysuria or urethral discharge in the past but that it had resolved spontaneously. The others denied having any symptoms.

Thus among asymptomatic men with condylomata acuminata 34% had asymptomatic urethral gonococcal infection. Patients with other sexually transmitted diseases, such as genital herpes and phthirias pubis, most probably also had a high incidence of asymptomatic gonorrhoea.

The finding of one sexually transmitted disease is a clear indication that another may also be present. As there has been a

steady increase in the incidence of gonorrhoea, the existence of this disease should be suspected. Even though a patient has no symptoms of gonorrhoea, smears and cultures from the anterior urethra should be carried out in the interest of personal as well as public health.

Yours faithfully,
Marwali Harahap

Department of Dermatovenereology,
University of North Sumatra, Medical
School,
Medan,
Indonesia

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Correction

Preservation of *Neisseria gonorrhoeae* by the gelatin-disc method

In the paper by S. Yamai *et al.* (April, 1979, p. 91) the fourth line should have read, '(2) Distilled water solution of sodium L-ascorbate (5%, Wako) . . .'

Book Review

Sexually Transmitted Diseases. R. C. Noble, 1979. Pp. 158 and index. Henry Kimpton, London (£6.00)

The author, who is an associate professor at the University of Kentucky, has written this book for physicians, house staff, and clinic personnel. It is a convenient size and shape for carrying in the pocket of a clinic coat and could be used for reference at work. Since the book is written from an American viewpoint, a review written by a British doctor, who is unfamiliar with the normal clinic management in America, may possibly be overcritical. I am sure that it would succeed in its objective in America, but as treatment schedules in America and Britain differ—particularly for gonorrhoea and syphilis—its place in British venereology is less assured. However, there are some excellent chapters in the book and I would suggest that it might be read with profit by venereologists in this country.

The opening chapter on patient management is one such chapter. Useful tables of differential diagnoses are given, and a section is contributed by a lady physician on the 'consumer viewpoint' in pelvic examinations. The author uses a precise style of prose, almost like lecture notes, which has the virtue of imparting information concisely.

The chapter on gonococcal infections covers the entire spectrum of diseases caused by the gonococcus. Disseminated gonococcal infection is almost over-

emphasised, but there is no mention of blood cultures or of the gonococcal complement fixation test in this condition. In the section on diagnosis the modified Thayer-Martin medium is stressed as the ideal culture medium, but there is no mention of Stuart's or Amies's transport media. Urethral cultures from female patients are only recommended after hysterectomy, rectal cultures are taken blind in both men and women, and proctoscopy is not performed routinely on male homosexuals. In the section on treatment, there is no mention of the Lomholt and Olsen regimen using benzyl penicillin and probenecid, presumably as the only therapeutic regimens recommended are those suggested by the Center for Disease Control. The number of post-treatment follow-up visits recommended is fewer than in British clinics. One can only assume that two-glass urine tests are not performed in America.

The chapter on syphilis is again very thorough and there are clear instructions on the techniques for obtaining specimens for darkground examinations from chancres and lymph nodes. Serological tests are clearly explained. The author is a firm believer in epidemiological treatment for both syphilis and gonorrhoea, although few venereologists in this country would use the treatments recommended. The problems of syphilis in pregnancy and congenital syphilis are discussed. Although congenital syphilitic deafness is mentioned, its treatment with steroids is not.

The other chapters in the book are shorter but deal with the other sexually transmitted diseases individually in great detail. Topics not commonly dealt with in British textbooks are *Corynebacterium vaginale* vaginitis and the 'gay-bowel' syndrome, both of which chapters are worth reading. The chapter on pediculosis deals with all forms of louse infestation. The pharmacology of metronidazole is very thoroughly described, but its use in the first trimester of pregnancy is not recommended; whether the alternative (a weak vinegar douche) would be acceptable in Britain is debatable. The chapter on herpes simplex is also worth reading.

The author states in the preface that he deliberately omitted Reiter's syndrome and Behçet's syndrome. Another omission is balanitis xerotica obliterans.

The one serious flaw in the book is the illustrations. These are too small, unbalanced in selection (there are seven of the skin rash in disseminated gonococcal infection but only three in the chapter on syphilis), and some plates simply lack the contrast necessary to clarify the relevant points in monochrome plates. Hopefully the users of the book would work in busy departments with adequate clinical material to remedy this.

References are provided for each chapter from both American and British journals to encourage further reading.

G. D. Morrison

Index to Volume 55, 1979

Authors and subjects are indexed together. There is a separate index of articles abstracted and another of authors of articles abstracted.

- ABBARO, S. A. See TAHA, O. M. A., *et al.*, 313
ADEYEMI-DORO, F. A. B., OSOBA, A. O., and JUNAID, T. A.: Peri-genital cutaneous schistosomiasis, 446
ADLER, M. W. See O'CONNOR, B. H., *et al.*, 52
AHMED, M. A. See TAHA, O. M. A., *et al.*, 313
AHO, K. See PAAVONEN, J., *et al.*, 203
AHRONS, S. See MÖLLER, B. R., *et al.*, 422
AMARASURIYA, K. L.: Haematuria presenting in outpatients attending a department of genitourinary medicine, 214
ALDEN, J. See GRAVES, S.,
AL RAWI, Z. H. See THIN, R. N., *et al.*, 348
Amoebiasis, epidemic in biased population, 375
Amoxycillin and ampicillin plus probenecid, trial of, in treatment of gonorrhoea in men, 340
Amoxycillin, effect of probenecid, 211
Ampicillin plus probenecid and amoxycillin, trial of, in treatment of, gonorrhoea in men, 340
Ampicillin versus minocycline in gonococcal urethritis, 411
ANDREW, C. W. See BALSDON, M. J., *et al.*, 289
ANONG, C. N. See SOGBETUN, A. O., *et al.*, 44
AUMONIER, F. J. A note on microscopy, 109
Australia, South, epidemiology of syphilis and gonorrhoea in, 131
Australian conference on STD, 304
Austria, routine screening of STDs over six years, 147
Autoanalyser, use in treatment monitoring of syphilis, 173
BABU, J. P. See SCHELL, R. F., *et al.*, 316
BACON, P. M.: Social distribution of sexually transmitted diseases, 295
BAIN, S. S. R. See McMILLAN, A., *et al.*, 5, 265
Balanitis, necrotising, due to herpes, 48
BALDETORP, B. See MÅRDH, P.-A. *et al.*, 256
BALSDON, M., GREEN, N., ANDREW, C. W., and JACKSON, D. H.: Rapid staining technique for *T. vaginalis*, 289
BARBHAIYA, R., THIN, R. N., TURNER, P., and WADSWORTH, J.: Clinical pharmacological studies of amoxycillin: effect of probenecid, 211
BARLOW, D. See WILLCOX, J. R., *et al.*, 281
BARROW, J. See WILLCOX, J. R., *et al.*, 281
BAUGHN, R. E. See LAPUSHIN, R. W., *et al.*, 168
BILLINGTON, T. See GRAVES, S.
BIRRY, A. and KASATIYA, S.: Evaluation of microhaemagglutination assay to determine treponemal antibodies in CSF, 239
BJERRE, B. See PERSSON, K., *et al.*, 429
BOOK REVIEWS
ARNDT, K. A.: Manual of Dermatologic Therapeutics with Essentials of Diagnosis, 2nd edition (1978), 306
BROOKS, G. F., GOTSCHLICH, E. C., HOLMÉS, K. K., SAWYER, W. D. and YOUNG, F. E. (Editors): Immunology of *N. gonorrhoeae* (1978), 306
GREITHER, A.: Dermatology and Venereology (1978), 231
NATIONAL HEALTH AND MEDICINE RESEARCH COUNCIL: Handbook on Sexually Transmitted Diseases (1978), 381
NOBLE, R. C.: Sexually Transmitted Diseases (1979), 451
ROBERTS, R. B. (Editor): The Gonococcus (1977), 231
SCHOFIELD, C. B. S.: Sexually Transmitted Diseases (1979), 381
SEHGAL, V. N.: A Textbook of Venereal Diseases (1978), 231
TRIMMER, E.: Basic Sexual Medicine (1978), 231
BOOKER, R. See THIN, R. N., *et al.*, 354
BOTH, P. See JESSOP, S., 292
BRATHWAITE, A. R.: Double-blind trial of amoxycillin and ampicillin plus probenecid in the treatment of gonorrhoea in men, 340
BRAYSON, J. See WAUGH, M. A., *et al.*,
BRUNINGS, E. A. See MENKE, H. E., *et al.*, 102
BRUNINGS, E. A. See NIEMEL, P. L. A., *et al.*, 99
Campylobacter jejuni in a male homosexual, 380
Campylobacter species in male homosexuals, 66
Candidosis, diagnosis, treatment and reporting of, 52
Candidosis, vulvovaginal, epidemiological investigation of patients with, 357
CAREY, P. B., and WRIGHT, E. P.: *Campylobacter jejuni* in a male homosexual (correspondence), 380
CATTERALL, R. D.: Sexually transmitted diseases in Ontario, 300
Cervix, secretory antibody response of, to infection with *N. gonorrhoeae*, 265
CHAN, J. K. See SCHELL, R. F., *et al.*, 316
CHAN, S. H., TAN, T., KAMARUDIN, A., WEE, G. B., and RAJAN, V. S.: HLA and sexually transmitted diseases in prostitutes, 207
Chancroid, rising incidence in Rotterdam, 439
CHIEF MEDICAL OFFICER: Sexually transmitted diseases (extract from Annual Report for 1977), 225
Chlamydiae, growth and effect of, in oviduct organ cultures, 194
Chlamydial culture service, need for, 281
Chlamydia trachomatis, antibodies to, in acute salpingitis, 26
C. trachomatis genital infections, 415
C. trachomatis in acute salpingitis, 203
C. trachomatis infection of the Fallopian tubes, 422
Ciliated epithelia of the human genital tract, 256
CLARK, A. See MELLERSH, A., *et al.*, 20
COOK, S. See THIN, R. N., *et al.*, 354
COOKE, E. M. See WAUGH, M. A., *et al.*, 411
COOPER, R. M. See NOBLE, R. C., *et al.*, 14, 336
CORRESPONDENCE
CAREY, P. B., and WRIGHT, E. P.: *Campylobacter jejuni* in a male homosexual, 380

- DEHERAGODA, P.: Anal canal versus rectal wall sampling, 230
- EVANS, B.: Management of non-specific urethritis, 305
- HARAHAP, M.: Asymptomatic gonorrhoea among patients with condylomata acuminata, 450
- MENKE, H. E., SCHULLER, J. L., STOLZ, E., NIEMEL, P. L. A., and MICHEL, M. F.: Treatment of lymphogranuloma venereum with rifampicin, 379
- NIA, A. K., and SMITH, E. L.: Pityriasis versicolor of glans penis, 230
- PAREEK, S.: Treatment of condyloma acuminatum with 5% 5-fluorouracil, 65
- PLATT, D. J., and GERKEN, A.: Inhibition of gonococci by a selective medium: disparity between isolates from sexual partners, 65
- SIMMONS, P. D., and TABAQCHALI, S.: *Campylobacter* species in male homosexuals, 66
- TREWARtha, F., and GRAVES, S.: Preparation of *T. pallidum* extracts from infected rabbit testes, 379
- WRIGHT, D. J. M., and FROST, D. J.: Possible suppressor T-lymphocyte activity in experimental syphilis, 66
- YOXALL, J. H.: Effect of allergy on response to treatment of non-gonococcal urethritis, 305
- COUFALIK, E. D. See TAYLOR-ROBINSON, D., et al., 30
- COUFALIK, E. D., TAYLOR-ROBINSON, D., and CSONKA, G. W.: Treatment of non-gonococcal urethritis with rifampicin as a means of defining the role of *Ureaplasma urealyticum*, 36
- CRISWELL, B. S. See GOMEZ, C. I., et al., 245
- CRUCE, D. D. See HUNTER, E. F., et al., 105
- CSF, evaluation of microhaemagglutination assay to determine treponemal antibodies in, 239
- CSONKA, G. W. See COUFALIK, E. D., et al., 37
- CSONKA, G. W. See TAYLOR-ROBINSON, D., et al., 30
- DARUGAR, S. See SIMMONS, P. D., et al., 419
- DARUGAR, S. See THAMBAR, I. V., et al., 284
- DARUGAR, S. See TREHARNE, J. D., et al., 26
- DEHERAGODA, P.: Anal canal versus rectal wall sampling (correspondence), 230
- DOURMASHKIN, R. R. See HUTCHINSON, G. R., et al., 194
- Doxycycline, oral, effect on syphilis, 110
- EGGLESTONE, S. I. See MORRISON, G. D., et al., 320
- ELLINGER, A. See GEBHART, W., et al., 83
- Epidemiological investigation of patients with vulvovaginal candidosis, 357
- Epidemiology of gonorrhoea, a theory of, 58
- EVANS, B.: Management of non-specific urethritis (correspondence), 305
- EVANS, R. T. See TAYLOR-ROBINSON, D., et al., 30
- FANTA, D., See SÖLTZ-SZÖTS, J., et al., 123
- FANTA, D., GEBHART, W., GROSS, W., KOKOSCHKA, E. M., SÖLTZ-SZÖTS, J., and THURNER, J.: Results of routine screening of STDs in Austria over a six-year period, 147
- FELTON, W. F.: A theory of the epidemiology of gonorrhoea, 58
- FISK, P. G. See WILLCOX, J. R., et al., 281
- FIUMARA, N. J., and SIMKIN, A. D.: Reinfection with disseminated gonococcal disease: a case report, 223
- 5-Fluorouracil, treatment of genital warts with, 65
- FORSGREN, A. See PERSSON, K., et al., 429
- FORSGREN, A., and FORSSMAN, L.: Metronidazole-resistant *Trichomonas vaginalis*, 351
- FORSEY, T. See SIMMONS, P. D., et al., 419
- FORSSMAN, L. See FORSGREN, A., 351
- FRITZ, H. See MÅRDH, P.-A., et al., 256
- FROST, D. J., See WRIGHT, D. J. M., et al., 66
- FTA-ABS test, double staining procedure for, 105
- GEBHART, W. See FANTA, D., et al., 147
- GEBHART, W., JURECKA, W., SÖLTZ-SZÖTS, J., THURNER, J., ELLINGER, A., and KITZ, K.: Effect of different preparation procedures on the ultrastructure of gonococci, 83
- GEDDE-DAHL, T. W. See ØDEGAARD, K., 334
- Gelatin disc method, preservation of *N. gonorrhoeae* using, 90
- GERKEN, A. See PLATT, D. J., et al., 65
- Germany, W., sensitivity to penicillin of *N. gonorrhoeae*, 80
- GHADIRIAN, F. D., and ROBSON, H. G.: *Chlamydia trachomatis* genital infections, 415
- Giardiasis, epidemic in biased population, 375
- GILES, A. J. H., and LAWRENCE, A. G.: Treatment failure with penicillin in early syphilis, 62
- GOMEZ, C. I., STENBACK, W. A., JAMES, A. N., CRISWELL, B. S., and WILLIAMS, R. P.: Attachment of *N. gonorrhoeae* to human sperm: microscopical study of effect of trypsin and iron, 245
- Gonococcal pharyngeal infection, misdiagnosis of meningococcal colonisation, 336
- Gonococcal urethritis, comparison of ampicillin and minocycline, 411
- Gonococcal urethritis, rapid diagnosis using limulus lysate test, 179
- Gonococcal urethritis, single-dose minocycline in treatment of, 343
- Gonococci, effect of preparation procedures on ultrastructure of, 83
- Gonococci, failure of iron to promote attachment of human sperms to, 329
- Gonococci, inhibition by selective medium: disparity between isolates from sexual partners, 65
- Gonorrhoea, asymptomatic, in patients with genital warts, 450
- Gonorrhoea, a theory of the epidemiology of, 58
- Gonorrhoea, diagnosis of, anorectal, comparing anal and rectal swabs, 186
- Gonorrhoea, diagnosis of, in women, 10
- Gonorrhoea, disseminated, reinfection with, 223
- Gonorrhoea, epidemiology, in New Zealand, 138
- Gonorrhoea, epidemiology, in South Australia, 131
- Gonorrhoea, how often sexually transmitted?, 278
- Gonorrhoea in men, trial of amoxycillin and ampicillin plus probenecid, 340
- Gonorrhoea in women in Scotland, 274
- Gonorrhoea, rectal and pharyngeal infection in women in Sheffield, 408
- Gonorrhoea, sampling, anal canal versus rectal wall, 230
- Gonorrhoea, serum immunoglobulin response in uncomplicated, 5
- Gonorrhoea, short-term treatment of, with i.m. and oral forms of trimethoprim-sulphamethoxazole, 24

- Gonorrhoea surveillance, does it pay?, 142
 Gonorrhoea, treatment with mezlocillin, 191
 GRAVES, S. See TREWARTHA, F., 379
 GRAVES, S.: Susceptibility of rabbits to *Treponema pallidum* after infection with *Mycobacterium bovis*, 394
 GRAVES, S., and ALDEN, J.: Limited protection of rabbits against infection with *Treponema pallidum* by immune rabbit sera, 399
 GRAVES, S., and BILLINGTON, T.: Optimum concentration of dissolved oxygen for the survival of virulent *Treponema pallidum* under conditions of low oxidation-reduction potential, 387
 GREEN, N. See BALSODN, M. J., *et al.*, 289
 GROSS, W. See FANTA, D., *et al.*, 147
 GRUNDER, K. See PETZOLDT, D., *et al.*, 80
 GYORKEY, P. See LAPUSHIN, R. W., *et al.*, 168
- Haematuria in patients attending a department of genito-urinary medicine, 214
 HAFIZ, S. See MELLERSH, A., *et al.*, 20
 HAG ALI M. See TAHA, O. M. A., *et al.*, 313
 HÅKANSSON, C. H. See MÅRDH, P.-A., *et al.*, 256
 Hamsters, CB, use in study of *T. pertenue*, 316
 Hamsters, golden, cultivation of *T. pallidum* in sub-cutaneous chambers in, 320
 HANSSON, H. See PERSSON, K., *et al.*, 429
 HARAHAP, M.: Asymptomatic gonorrhoea among patients with genital warts (correspondence), 450
 HARRIS, A. B. See YOUNG, H., *et al.*, 188
 Harrison Lecture 1978, 161
 HARRISON, R. M.: Illness behaviour and STDs, 125
 HENRIKSON, H. See MÖLLER, B. R., *et al.*, 422
 Hepatitis B surface antigen, follow up of patients with, 116
 Hepatitis B surface antigen, sexual transmission of, 366
 Herpes, genital, therapeutic aspects, 123
 Herpes, genital, vaccination for, 121
 Herpes simplex virus type 2, necrotising balanitis due to, 48
 Herpesvirus hominis antibodies among children and young adults in Ibadan, 44
 HILTON, A. L. See WARNOCK, D. W., *et al.*, 357
 HLA and sexually transmitted diseases in prostitutes, 207
 Homosexuals, *Campylobacter jejuni* in, 380
 Homosexuals, *Campylobacter* species in, 66
 HOVELIUS, B., THELIN, I., and MÅRDH, P.-A.: *Staphylococcus saprophyticus* in the aetiology of non-gonococcal urethritis, 369
 HUNTER, E. F., MCKINNEY, R. M., MADDISON, S. E., and CRUCE, D. D.: Double-staining procedure for FTA-ABS test, 105
 HUTCHINSON, G. R., TAYLOR-ROBINSON, D., and DOURMASHKIN, R. R.: Growth and effect of chlamydiae in human and bovine oviduct organ cultures, 194
 Hysterectomy, effect on genital infections, 434
- Ibadan, herpesvirus hominis antibodies among children and young adults in, 44
 Immunoglobulins serum, response in uncomplicated gonorrhoea, 5
 INABA, N., OHKAWA, R., MATSUURA, A., KUDOH, J., and TAKAMIZAWA, H.: Sexual transmission of hepatitis B surface antigen, 366
 Iron, effect of, on attachment of *N. gonorrhoeae* to sperm, 245
 Iron, failure to promote attachment of gonococci to human sperms, 329
 IUVDI: Bulletin, 29th General Assembly Leeds, 21-25 May 1978, 75
- JACKSON, D. H. See BALSODN, M. J., *et al.*, 289
 JAMES, A. N. See GOMEZ, C. I., *et al.*, 245
 JESSOP, S., and BOTHA, P.: False-positive results for syphilis in relatives of a patient with systemic lupus erythematosus, 292
 JOHNSON, A. P., and OSBORN, M. F.: Failure of iron to promote attachment of gonococci to human spermatozoa under physiological conditions, 329
 JOHNSON, T. See PERSSON, K., *et al.*, 429
 JORGENSEN, D.: Epidemiology of gonorrhoea and syphilis in South Australia (1966-77), 131
 JUDSON, F. N., and RUDER, M. A.: Effect of hysterectomy on genital tract infections, 434
 JUNAID, T. A. See ADEYEMI-DORO, F. A. B., *et al.*, 446
 JURECKA, W. See GEBHART, W., *et al.*, 83
- KAMARUDIN, A. See CHAN, S. H., *et al.*, 207
 KASATIYA, S. See BIRRY, S., 239
 KEAN, B. H., WILLIAM, D. C., and LUMINAIS, S. K.: Epidemic of amoebiasis and giardiasis in a biased population, 375
 KERSHAW, P. I. See WARNOCK, D. W., *et al.*, 357
 KINGHORN, G. R., and RASHID, S.: Prevalence of rectal and pharyngeal infection in women with gonorrhoea in Sheffield, 408
 KITZ, K. See GEBHART, W., *et al.*, 83
 KNOX, J. M.: Group B streptococcal infection, 118
 KOKOSCHKA, E. M. See FANTA, D., *et al.*, 147
 KOLATOR, B. See SETH, A. D., *et al.*, 325
 KOLATOR, B., and RODIN, P.: Comparison of anal and rectal swabs in diagnosis of anorectal gonorrhoea in women, 186
 KUDOH, J. See INABA, N. *et al.*, 366
- LANGLET, F. See SIMMONS, P. D., *et al.*, 419
 LANGLET, F. See THIN, R. N., *et al.*, 354
 LAPUSHIN, R. W., BAUGHN, R. E., MUSHER, D. M., and GYORKEY, P.: Ultrastructural study of satellite lymph nodes in syphilitic rabbits, 168
 LASSUS, A., and RENKONEN, O.-V.: Mezlocillin in the treatment of gonorrhoea, 191
 LASSUS, A., and RENKONEN, O.-V.: Short-term treatment of gonorrhoea with i.m. and oral forms of trimethoprim-sulphamethoxazole, 24
 LAWRENCE, A. G. See GILES, A. J. H., *et al.*, 62
 LE FROCK, J. L. See SCHELL, R. F., *et al.*, 316
 Limulus lysate test, rapid diagnosis of gonococcal urethritis, 179
 London, sensitivity to antibiotics of *N. gonorrhoeae* in, (1976-78), 325
 LUMINAIS, S. K. See KEAN, B. H., *et al.*, 375
 Lymph nodes, ultrastructural study of, in syphilitic rabbits, 168
 Lymphogranuloma venereum, treatment with rifampicin, 379
- MacFARLANE, D. E., and McMILLAN, A.: Treatment monitoring in syphilis using the autoanalyser, 173
 MCKINNEY, R. M., See HUNTER, E. F., *et al.*, 105

- McMILLAN, A. See MacFARLANE, D. E., *et al.*, 173
- McMILLAN, A., McNEILLAGE, G., YOUNG, H., and BAIN, S. R.: Serum immunoglobulin response in uncomplicated gonorrhoea, 5
- McMILLAN, A., McNEILLAGE, G., YOUNG, H., and BAIN, S. R.: Secretory antibody response of the cervix to infection with *N. gonorrhoeae*, 265
- McMILLAN, A., and PATTMAN, R. S.: Evaluation of urethral culture for *N. gonorrhoeae* in the routine investigation of men attending an STD clinic, 271
- McNEILLAGE, G. See McMILLAN, A., *et al.*, 5, 265
- MADDISON, S. E. See HUNTER, E. F., *et al.*, 105
- MÄRDH, P.-A. See HOVELIUS, B., *et al.*, 369
- MÄRDH, P.-A. See MÖLLER, B. R., *et al.*, 422
- MÄRDH, P.-A. See TREHARNE, J. D., *et al.*, 26
- MÄRDH, P.-A., BALDETORP, B., HÅKANSSON, C. H., FRITZ, H., and WESTRÖM, L.: Studies of ciliated epithelia of the human genital tract, 256
- MALLON, R. See MUNRO, R., 404
- MATSUURA, A. See INABA, N., *et al.*, 366
- MELLERSH, A., CLARK, A., and HAFIZ, S.: Inhibition of *N. gonorrhoeae* by normal human saliva, 20
- Meningococcal pharyngeal colonisation, misdiagnosis as gonococcal pharyngeal infection, 336
- MENKE, H. E. See NIEMEL, P. L. A., *et al.*, 99
- MENKE, H. E., SCHULLER, J. L., STOLZ, E., NIEMEL, P. L. A., and MICHEL, M. F.: Treatment of lymphogranuloma venereum with rifampicin (correspondence), 379
- MENKE, H. E., VELDKAMP, J., BRUNINGS, E. A., NIEMEL, P. L. A., NOTOWICZ, A., and STOLZ, E.: Comparison of cardiolipin and treponemal tests in serodiagnosis of yaws, 102
- Metronidazole, double-blind comparison of single dose and 5-day course in treatment of trichomoniasis, 354
- Metronidazole-resistant *T. vaginalis*, 351
- METZGER, M.: Role of humoral versus cellular resistance in pathogenesis of syphilis, 94
- Mezlocillin in treatment of gonorrhoea, 191
- MICHEL, M. F. See MENKE, H. E., *et al.*, 379
- MICHEL, M. F. See NAYYAR, K. C., *et al.*, 439
- Microcult-GC culture test, an assessment of the, 1
- Microhaemagglutination assay to determine treponemal antibodies in CSF, 239
- Microscopy, note on, 109
- MILLER B. R. See NOBLE, R. C., *et al.*, 14
- MILNE, J. D. See WARNOCK, D. W., *et al.*, 357
- MILNE, J. D., and WARNOCK, D. W.: Effect of simultaneous oral and vaginal treatment on the rate of cure and relapse in vaginal candidosis, 362
- Minocycline, single-dose, in treatment of gonococcal urethritis, 343
- Minocycline versus ampicillin in gonococcal urethritis, 411
- MIYAMOTO, Y. See YAMAI, S., *et al.*, 90
- MÖLLER, B. R., MÄRDH, P.-A., WESTRÖM, L., AHRONS, S., RIPA, K. T., SVENSSON, L., VON MECKLENBURG, C., and HENRIKSON, H.: *Chlamydia trachomatis* infection of the Fallopian tubes, 422
- MONTEFIORE, D. See SOGBETUN, A. O., *et al.*, 44
- MORRISON, G. D., EGGLESTONE, S. I., and NORTHWOOD, J. L.: Cultivation of *T. pallidum* in subcutaneous chambers implanted in golden hamsters, 320
- MORTON, R. S.: Australian conference on STD in Perth, Western Australia, August 1978, 304
- MSSVD, annual report, 68
- MUNDAY, P. E. See TAYLOR-ROBINSON, D., *et al.*, 30
- MUNRO, R., and MALLON, R.: Reverse passive haemagglutination test for the rapid identification of *Neisseria gonorrhoeae* and detection of penicillinase production, 404
- MUSHER, D. M. See LAPUSHIN, R. W., *et al.*, 168
- Mycobacterium bovis*, susceptibility of rabbits to *T. pallidum* after infection with, 394
- Mycoplasma hominis* in chlamydial and non-chlamydial nongonococcal urethritis, 30
- NASEMANN, TH., and WASSILEW, S. W.: Vaccination for herpes simplex genitalls, 121
- NAYYAR, K. C., STOLZ, E., and MICHEL, M. F.: Rising incidence of chancroid in Rotterdam, 439
- NEHAUL, B. B. G. See WAUGH, M. A., *et al.*, 411
- Neisseria gonorrhoeae* and *N. meningitidis*, frequency of simultaneous carriage, 334
- N. gonorrhoeae*, assessment of selective medium for, 183
- N. gonorrhoeae*, attachment to human sperm, effect of trypsin and iron on, 245
- N. gonorrhoeae*, inhibition by human saliva, 20
- N. gonorrhoeae*, pharyngeal colonisation in black and white patients, 14
- N. gonorrhoeae*, preservation by gelatin disc method, 90
- N. gonorrhoeae*, reverse passive haemagglutination test for, 404
- N. gonorrhoeae*, secretory antibody response of cervix to infection with, 265
- N. gonorrhoeae*, sensitivity to antibiotics in London 1976-78, 325
- N. gonorrhoeae*, sensitivity to penicillin in W. Germany, 80
- N. gonorrhoeae*, urethral culture for, in men attending an STD clinic, 271
- N. meningitidis* and *N. gonorrhoeae*, frequency of simultaneous carriage, 334
- N. meningitidis*, pharyngeal colonisation in black and white patients, 14
- Neisserial infection, individual susceptibility?, 188
- NEUBERT, U. See PETZOLDT, D., *et al.*, 80
- New Zealand, epidemiology of syphilis and gonorrhoea in, 138
- NIA, A. K., and SMITH, E. L.: Pityriasis versicolor of glans penis (correspondence), 230
- NICOL, C. S.: The Harrison Lecture 1978: Sexually transmitted diseases and travel, 161
- NIEMEL, P. L. A. See MENKE, H. E., *et al.*, 102, 379
- NIEMEL, P. L. A., BRUNINGS, E. A., and MENKE, H. E.: Attenuated yaws in Surinam, 99
- NIKKAWA, T. See YAMAI, S., *et al.*, 90
- NOBLE, R. C., and COOPER, R. M.: Meningococcal colonisation misdiagnosed as gonococcal pharyngeal infection, 336
- NOBLE, R. C., COOPER, R. M., and MILLER B. R.: Pharyngeal colonisation by *N. gonorrhoeae* and *N. meningitidis* in black and white patients attending a venereal disease clinic, 14
- Nongonococcal urethritis, comparison of seven-day and 21-day courses of triple tetracycline, 284
- Nongonococcal urethritis, concealed, epidemiological importance of, 149

- Nongonococcal urethritis, effect of allergy on response to treatment, 305
- Nongonococcal urethritis, *Staphylococcus saprophyticus* in aetiology of, 369
- Nongonococcal urethritis, treatment with rifampicin to define the role of *Ureaplasma urealyticum*, 36
- Nongonococcal urethritis, *Ureaplasma urealyticum* and *Mycoplasma hominis* in chlamydial and nonchlamydial, 30
- Non-specific urethritis, management of, 305
- NORTHWOOD, J. L. See MORRISON, G. D., *et al.*, 320
- NOTOWICZ, A. See MENKE, H. E., *et al.*, 102
- OATES, J. K. See TAYLOR-ROBINSON, D., *et al.*, 30
- OBARA, Y. See YAMAI, S., *et al.*, 90
- O'CONNOR, B. H., and ADLER, M. W.: Current approaches to the diagnosis, treatment and reporting of trichomoniasis and candidosis, 52
- ØDEGAARD, K., and GEDDE-DAHL, T. W.: Frequency of simultaneous carriage of *N. gonorrhoeae* and *N. meningitidis*, 334
- OHKAWA, R. See INABA, N., *et al.*, 366
- OMER, E. E. See TAHA, O. M. A., *et al.*, 313
- ONODA, Y.: Therapeutic effect of oral doxycycline on syphilis, 110
- Ontario, STD in, 300
- ORIEL, J. D. See WATERWORTH, P. M., *et al.*, 343
- OSBORN, M. F. See JOHNSON, A. P., 329
- OSOBA, A. O. See ADEYEMI-DORO, F. A. B., *et al.*, 446
- Oxytetracycline concentrations, vaginal, 348
- PAAVONEN, J., SAIKKU, P., VESTERINEN, E., and AHO, K.: *Chlamydia trachomatis* in acute salpingitis, 203
- PACKER, H. See RENDTORFF, R. C., *et al.*, 142
- PANDHI, P. K. See SIMMONS, P. D., *et al.*, 419
- PAREEK, S.: Treatment of condyloma acuminatum with 5% 5-fluorouracil (correspondence), 65
- PARTON, C. W., and SGROI, S. M.: An alternative to the problem of insufficient physician manpower in the clinical care of STDs, 144
- PATTMAN, R. S. See McMILLAN, A., 271
- Penicillin treatment failure in early syphilis, 62
- Penile pityriasis versicolor, 230
- PERKINS, R. L. See SPAGNA, V. A., *et al.*, 179
- PERSSON, K., PERSSON, K., HANSSON, H., BJERRE, B., SVANBERG, L., JOHNSSON, T., and FORSGREN, A.: Prevalence of nine different micro-organisms in the female genital tract, 429
- PERSSON, K., See PERSSON, K., *et al.*, 429
- PETZOLDT, D., GRUNDER, K., and NEUBERT, U.: Sensitivity of *Neisseria gonorrhoeae* to penicillin in West Germany, 80
- PEUTHERER, J. F., SMITH, I. W., and ROBERTSON, D. H. H.: Necrotising balanitis due to a generalised primary infection with herpes simplex virus type 2, 48
- Pharyngeal colonisation by *N. gonorrhoeae* and *N. meningitidis* in black and white patients attending a venereal disease clinic, 14
- PHILLIPS, I. See TAYLOR, E., *et al.*, 183
- PHILLIPS, I. See UNSWORTH, P. F., *et al.*, 1
- Pityriasis versicolor of glans penis, 230
- PLATT, D. J., and GERKEN, A.: Inhibition of gonococci by a selective medium: disparity between isolates from sexual partners (correspondence), 65
- PLATTS, W. M.: Epidemiology of gonorrhoea and syphilis in New Zealand, 138
- Poland, evaluation of VD control campaign in, 127
- PRENTICE, M. J. See TAYLOR-ROBINSON, D., *et al.*, 30
- PRIOR, R. B. See SPAGNA, V. A., *et al.*, 179
- Probenecid, effect on amoxycillin, 211
- Prostitutes, HLA and STDs in, 207
- RAJAN, V. S. See CHAN, S. H., *et al.*, 207
- RASHID, S. See KINGHORN, G. R., 408
- RENDELL, P. See THIN, R. N., *et al.*, 278
- RENDTORFF, R. C., and PACKER, H.: Does gonorrhoea surveillance pay?, 142
- RENKONEN, O.-V. See LASSUS, A., *et al.*, 24, 191
- RIDGWAY, O. L. See WATERWORTH, P. M., *et al.*, 343
- Rifampicin in treatment of lymphogranuloma venereum, 379
- Rifampicin, treatment of nongonococcal urethritis with, to define role of *Ureaplasma urealyticum*, 36
- RIPA, K. T. See MØLLER, B. R., *et al.*, 422
- RIPA, K. T. See TREHARNE, J. D., *et al.*, 26
- ROBERTSON, D. H. H. See PEUTHERER, J. F., *et al.*, 48
- ROBERTSON, D. H. H. See YOUNG, H., *et al.*, 188
- ROBSON, H. G. See GHADIRIAN, F. D., 415
- RODIN, P. See KOLATOR, B., *et al.*, 186
- Rotterdam, rising incidence of chancroid in, 439
- RUDER, M. A. See JUDSON, F. N.
- SAIKKU, P. See PAAVONEN, J., *et al.*, 203
- Saliva, normal human, inhibition of *N. gonorrhoeae* by, 20
- Salpingitis, acute, antibodies to *Chlamydia trachomatis* in, 26
- Salpingitis, acute, *Chlamydia trachomatis* in, 203
- SCHELL, R. F., LE FROCK, J. L., BABU, J. P., and CHAN, J. K.: Use of CB hamsters in the study of *T. pertenuis*, 316
- Schistosomiasis, perigenital cutaneous, 446
- SCHOFIELD, C. B. S.: Gonorrhoea in women in Scotland, 274
- SCHOFIELD, C. B. S.: Seasonal variations in the reported incidence of sexually transmitted diseases in Scotland (1972-1976), 218
- SCHULLER, J. L. See MENKE, H. E., *et al.*, 379
- Scotland, gonorrhoea in women in, 274
- Scotland, seasonal variations in STDs, 218
- SETH, A. D., KOLATOR, B., and WILKINSON, A. E.: Sensitivity of *N. gonorrhoeae* to antibiotics in London (1976-78), 325
- SGROI, S. M. See PARTON, C. W., *et al.*, 144
- SHAW, E. J. See THIN, R. N., *et al.*, 10
- Sheffield, rectal and pharyngeal infection with gonorrhoea in, 408
- SHIMODA, Y. See YAMAI, S., *et al.*, 90
- SIMKIN, A. D. See FIUMARA, N. J., *et al.*, 223
- SIMMONS, P. D. See THAMBAR, I. V., *et al.*, 284
- SIMMONS, P. D. See THIN, R. N., *et al.*, 348
- SIMMONS, P. D.: Follow up of patients with hepatitis B surface antigen, 116
- SIMMONS, P. D., FORSEY, T., THIN, R. N., TREHARNE, J. D., DAROUGAR, S., LANGLET, F., and PANDHI, P. K.: Anti-chlamydial antibodies in pelvic inflammatory disease 419
- SIMMONS, P. D., and TABAQCHALI, S.: *Campylobacter* species in male homosexuals (correspondence), 66

- SMITH, E. L. See NIA, A. K., *et al.*, 230
- SMITH, I. W. See PEUTHERER, J. F., *et al.*, 48
- SOGBETUN, A. O., MONTEFIORE D., and ANONG C. N.: Herpes-virus hominis among children and young adults in Ibadan, 44
- SÖLTZ-SZÖTS, J. See FANTA, D., *et al.*, 147
- SÖLTZ-SZÖTS, J. See GEBHART, W., *et al.*, 83
- SÖLTZ-SZÖTS, J., and FANTA, D.: Therapeutic aspects of herpes genitalis, 123
- SPAGNA, V. A., PRIOR, R. B., PERKINS, R. L.: Rapid presumptive diagnosis of gonococcal urethritis in men by the limulus lysate test, 179
- SPELLER, D. C. E. See WARNOCK, D. W., *et al.*, 357
- Sperm, human, attachment of *N. gonorrhoeae* to, effect of trypsin and iron, 245
- Sperms, human, failure of iron to promote attachment of gonococci to, 329
- Staphylococcus saprophyticus* in the aetiology of nongonococcal urethritis, 369
- STD and travel, 161
- STD, Australian conference on, 304
- STD, clinical care of, with insufficient physician manpower, 144
- STD, illness behaviour and, 125
- STD in Ontario, 300
- STD in patients attending VD clinics in Khartoum, Sudan, 313
- STD, routine screening of, in Austria over six years, 147
- STD, seasonal variations in Scotland, 218
- STD, social distribution of, 295
- STENBACK, W. A. See GOMEZ, C. I., *et al.*, 245
- STOLZ, E. See MENKE, H. E., *et al.*, 102, 379
- STOLZ, E. See NAYYAR, K. C., *et al.*, 439
- Streptococcal infection, group B, 118
- SUBRAMANIAN, S. See WATERWORTH, P. M., *et al.*, 343
- Sudan, STDs in patients attending VD clinics in, 313
- Surinam, attenuated yaws in, 99
- SVANBERG, L. See PERSSON, K., *et al.*, 429
- SVENSSON, L. See MÖLLER, B. R., *et al.*, 422
- SVENSSON, L. See TREHARNE, J. D., *et al.*, 26
- SYMONDS, M. A. E. See THIN, R. N., *et al.*, 354
- Syphilis, early, treatment failure with penicillin, 62
- Syphilis, effect of oral doxycycline on, 110
- Syphilis, epidemiology, in New Zealand, 138
- Syphilis, epidemiology, in South Australia, 131
- Syphilis, experimental, possible suppressor T-lymphocyte activity in, 66
- Syphilis, false-positive tests for, in relatives of a patient with systemic lupus erythematosus, 292
- Syphilis, role of humoral versus cellular resistance in pathogenesis, 94
- Syphilis, treatment monitoring, using autoanalyser, 173
- Syphilitic rabbits, ultrastructural study of lymph nodes in, 168
- Systemic lupus erythematosus, false-positive results for syphilis in relatives of a patient with, 292
- TABAQCHALI, S. See SIMMONS, P. D., *et al.*, 66
- TABAQCHALI, S. See THIN, R. N., *et al.*, 348
- TAHA, O. M. A., HAG, A. I. M., OMER, E. E., AHMED, M. A., and ABBARO, S. A.: Study of STDs in patients attending venereal disease clinics in Khartoum, Sudan, 313
- TAKAMIZAWA, H. See INABA, N., *et al.*, 366
- TALSANIA, H. See UNSWORTH, P. F., *et al.*, 1
- TAN, T. See CHAN, S. H., *et al.*, 207
- TAYLOR, E., and PHILLIPS, I.: Assessment of a selective medium for the isolation of *N. gonorrhoeae*, 183
- TAYLOR-ROBINSON, D. See COUFALIK, E. D., *et al.*, 36
- TAYLOR-ROBINSON, D. See HUTCHINSON, G. R., *et al.*, 194
- TAYLOR-ROBINSON, D., EVANS, R. T., COUFALIK, E. D., PRENTICE, M. J., MUNDAY, P. E., CSONKA, G. W., and OATES J. K.: *Ureaplasma urealyticum* and *Mycoplasma hominis* in chlamydial and non-chlamydial nongonococcal urethritis, 30
- Tetracycline, triple, comparison of seven- and 21-day courses in nongonococcal urethritis, 284
- THAMBAR, I. V., SIMMONS, P. D., THIN, R. N., DAROUGAR, S., and YEARSLEY, P.: Double-blind comparison of two regimens in the treatment of nongonococcal urethritis: seven-day versus 21-day courses of triple tetracycline (Deteclo), 284
- THELIN, I. See HOVELIUS, B., *et al.*, 369
- THIN, R. N. See BARBHAIYA, R., *et al.*, 211
- THIN, R. N. See SIMMONS, P. D., *et al.*, 419
- THIN, R. N. See THAMBAR, I. V., *et al.*, 284
- THIN, R. N., AL RAWI, Z. H., SIMMONS, P. D., TREHARNE, J. D., and TABAQCHALI, S.: Vaginal oxytetracycline concentrations, 348
- THIN, R. N., RENDELL, P., and WADSWORTH, J.: How often are gonorrhoea and genital yeast infection sexually transmitted?, 278
- THIN, R. N., and SHAW E. J.: Diagnosis of gonorrhoea in women, 10
- THIN, R. N., SYMONDS, M. A. E., BOOKER, R., COOK, S., and LANGLET, F.: Double-blind comparison of a single dose and a five-day course of metronidazole in the treatment of trichomoniasis, 354
- TURNER, J. See FANTA, D., *et al.*, 147
- TURNER, J. See GEBHART, W., *et al.*, 83
- TOWPIK, J.: Critical evaluation of venereal disease control campaign in Poland (1970-76), 127
- TREHARNE, J. D. See SIMMONS, P. D., *et al.*, 419
- TREHARNE, J. D. See THIN, R. N., *et al.*, 348
- TREHARNE, J. D., RIPA, K. T., MÄRDH, P.-A., SVENSSON, L., WESTRÖM, L., and DAROUGAR S.: Antibodies to *Chlamydia trachomatis* in acute salpingitis, 26
- Treponema pallidum* cultivation in subcutaneous chambers in golden hamsters, 320
- T. pallidum*, preparation of extracts from rabbits testes, 379
- T. pallidum*, susceptibility of rabbits to, after infection with *Mycobacterium bovis*, 394
- Treponema pertenue*, use of CB hamsters in study of, 316
- TREWARTHA, F., and GRAVES, S.: Preparation of *T. pallidum* extracts from infected rabbit testes (correspondence), 379
- Trichomonas vaginalis*, double-blind comparison of single dose and 5-day course in treatment of, 354
- T. vaginalis*, metronidazole-resistant, 351
- T. vaginalis*, rapid staining technique for, 289
- T. pallidum*, limited protection of rabbits against infection with, by immune rabbit sera, 399
- T. pallidum*, optimum concentration of dissolved oxygen for survival of, 387
- Trichomoniasis, diagnosis, treatment and reporting of, 52

- Trimethoprim-sulphamethoxazole, i.m. and oral forms in short-term treatment of gonorrhoea, 24
- Trypsin, effect of, on attachment of *N. gonorrhoeae* to sperm, 245
- TURNER, P. See BARBHAIYA, R., *et al.*, 211
- UNSWORTH, P. F., TALSANIA, H., and PHILLIPS I.: An assessment of the Microcult-GC culture test, 1
- Ureaplasma urealyticum* in chlamydial and non-chlamydial nongonococcal urethritis, 30
- U. urealyticum*, treatment of nongonococcal urethritis with rifampicin to define role of, 36
- Urethral culture of *N. gonorrhoeae* in men attending an STD clinic, 271
- Vaccination for genital herpes, 121
- Vaginal oxytetracycline concentrations, 348
- VD control campaign in Poland, 127
- VELDKAMP, J. See MENKE, H. E., *et al.*, 102
- VESTERINEN, E. See PAAVONEN, J., *et al.*, 203
- VON MECKLENBURG, C. See MÖLLER, B. R., *et al.*, 422
- WADSWORTH, J. See BARBHAIYA, R., *et al.*, 211
- WADSWORTH, J. See THIN, R. N., *et al.*, 278
- WARNOCK, D. W. See MILNE, J. D., 362
- WARNOCK, D. W., SPELLER, D. C. E., MILNE, J. D., HILTON, A. L., and KERSHAW, P. I.: Epidemiological investigation of patients with vulvovaginal candidosis, 357
- Warts, genital, in patients with asymptomatic gonorrhoea, 450
- Warts, genital, treatment with 5-fluorouracil, 65
- WASSILEW, S. W. See NASEMANN, TH., *et al.*, 121
- WATERWORTH, P. M., ORIEL, J. D., RIDGWAY, G. L., and SUBRAMANIAN, S.: Single-dose minocycline in the treatment of gonococcal urethritis, 343
- WAUGH, M. A., COOKE, E. M., NEHAUL, B. B. G., and BRAYSON, J.: Comparison of minocycline and ampicillin in gonococcal urethritis, 411
- WEE, G. B. See CHAN, S. H., *et al.*, 207
- WESTRÖM, L. See MÅRDH, P.-A., *et al.*, 256
- WESTRÖM, L. See MÖLLER, B. R., *et al.*, 422
- WESTRÖM, L. See TREHARNE, J. D., *et al.*, 26
- WILKINSON, A. E. See SETH, A. D., *et al.*, 325
- WILLCOX, J. R., FISK, P. G., BARROW, J., and BARLOW, D.: The need for a chlamydial culture service, 281
- WILLCOX, R. R.: Epidemiological importance of concealed nongonococcal urethritis, 149
- WILLIAM, D. C. See KEAN, B. H., *et al.*, 375
- WILLIAMS, R. P. See GOMEZ, C. I., *et al.*, 245
- Women, diagnosis of gonorrhoea in, 10
- WRIGHT, D. J. M., and FROST, D. J.: Possible suppressor T-lymphocyte activity in experimental syphilis (correspondence), 66
- WRIGHT, E. P. See CAREY, P. B., 380
- YAMAI, S., OBARA, Y., NIKKAWA, T., SHIMODA, Y., and MIYAMOTO, Y.: Preservation of *Neisseria gonorrhoeae* by the gelatin disc method, 90
- Yaws, attenuated, in Surinam, 99
- Yaws, comparison of cardiolipin and treponemal tests in serodiagnosis, 102
- YEARSLEY, P. See THAMBAR, I. V., *et al.*, 284
- Yeast infection, genital, how often sexually transmitted, 278
- YOUNG, H. See McMILLAN, A., *et al.*, 5, 265
- YOUNG, H., HARRIS, A. B., and ROBERTSON, D. H. H.: Individual susceptibility to neisserial infection?, 188
- YOXALL, J. H.: Effect of allergy on response to treatment of nongonococcal urethritis (correspondence), 305

Index to subjects of articles abstracted, 1979

*Indicates that only the title is given.

- Acridine orange stain in diagnosis of gonorrhoea, 384*
- Amphotericin B and minocycline, combined activity against yeasts, 157
- Amphotericin B, clotrimazole and miconazole, in-vitro studies, 238
- Ampicillin and co-trimoxazole compared in treatment of gonorrhoea, 236
- Ampicillin in pharyngeal gonorrhoea, 72
- Antibiotic penetration into cerebrospinal fluid and brain tissue, 74*
- Antichlamydial antibody in tears and sera in Tunisia, 157*
- Auxotypes and antibiotic susceptibility patterns from local and disseminated infections with *N. gonorrhoeae*, 308

- Bacampicillin, in gonorrhoea, 384
- Bacteriuria, asymptomatic, reliability of single urine culture in adult males, 386*
- Behcet's syndrome, a family study, 238*
- Behcet's syndrome, a family study, 386*
- Behcet's syndrome in pregnancy, 312*
- Bell's palsy and secondary syphilis, 307
- Benzylmetronidazole, single-dose treatment for *T. vaginalis*, 238*
- β -lactamase, contribution of a TEM-I-like, to penicillin resistance in *N. gonorrhoeae*, 71
- Buffy coats, value of looking at in febrile patients, 159*

- Candida*, recurrent vaginal infection, 157*
- Candida albicans*, correlation between serotype and susceptibility of 5-fluorocytosine, 385*
- C. albicans*, effect of 5-fluorocytosine on, 385*
- Candidal vaginitis, miconazole nitrate in, 385*
- Candidosis, chronic vaginal, cell-mediated deficiency and humoral immune response in, 238*
- Candidosis, vulvo-vaginal, in pregnancy, treated with clotrimazole, 310*
- Carcinoma *in-situ* changes in pigmented penile papules, 238*
- Cefoxitin sodium, effect on *N. gonorrhoeae*, 156
- Cefuroxime, in-vitro activity against *T. pallidum*, 70*
- Cerebrospinal fluid, penetration of antibiotics into, 74*
- Cervical carcinoma and herpes simplex virus type 2: animal model for induction of latency, 73*
- Cervical carcinoma, antibodies to herpes simplex virus type 2 in husbands of patients with, 73*
- Cervical carcinoma, organ culture model for herpes simplex virus type 2 in, 385
- Cervical carcinoma, relation of herpes simplex virus type 2 to, 385
- Cervicitis, chlamydiae and abnormal Papanicolaou smears, 310*
- Cervix, infection with *C. trachomatis*, 72
- Chancroid in Greenland 1977-78, 311
- Chimpanzee urethral and pharyngeal infections with *C. trachomatis*, 310
- Chlamydia* infection associated with Reiter's syndrome, 237
- Chlamydia* isolation methods in current medical practice and epidemiology, 73*
- Chlamydia trachomatis* and *N. gonorrhoeae* comparison of genital infections caused by, 237*
- C. trachomatis* and pneumonia in infants, two cases, 309
- C. trachomatis* cervicitis in gynaecological outpatients, 309
- C. trachomatis* cultures in mouse peritoneal macrophages: factors affecting growth, 73*
- C. trachomatis*, effect of cortisol on growth of, 156
- C. trachomatis*, examination for, in patients with non-gonococcal urethritis, 236
- C. trachomatis*, experimental chimpanzee urethral and pharyngeal infections, 310
- C. trachomatis*, experimental salpingitis in monkeys, 310*
- C. trachomatis* infection of cervix, 72
- C. trachomatis* infections, multiple site involvement in neonates, 309
- C. trachomatis*, 15-month follow-up in women infected with, 237
- C. trachomatis* nasopharyngitis and pneumonia in baboons, histopathology, 309
- C. trachomatis*, predicted efficacy of antibiotics in treatment of, 156
- C. trachomatis*, role in post-gonococcal urethritis in homosexual and heterosexual men, 308
- C. trachomatis*, sensitivity to rosamycin, 156
- C. trachomatis* urethritis, a culture and therapeutic study, 310*
- C. trachomatis*, vaccine against infection of genital tract with, 237*
- Chlamydiae, cervicitis and abnormal Papanicolaou smears, 310*
- Chlamydiae, immunoperoxidase and immunofluorescence staining for detection of, 157*
- Chlamydial antibodies in Crohn's disease and ulcerative colitis, 312
- Chlamydial antibodies, LGV-type in Crohn's disease, 311
- Chlamydial antibodies, single antigen immunofluorescence test for, 72
- Chlamydial infections of guinea pigs, enteric vaccines as protection against, 238
- Chlamydial ocular infections, rapid serological test for, 156
- Chlamydial pneumonia and human sexuality, 310*
- Chlamydial pneumonitis 73*, 309
- Chloramphenicol, inhibition of *N. gonorrhoeae*, 235*
- Cholesterol, binding by *N. gonorrhoeae*, 235*

- Clotrimazole in vulvo-vaginal candidosis in pregnancy, 310*
- Clotrimazole, miconazole and amphotericin B, in vitro studies, 238
- Congo-red disc, differentiation of *N. gonorrhoeae* and *N. meningitidis* by, 235
- Contagious equine metritis, serological response of patients with non-gonococcal urethritis to organism of, 310
- Cortisol, effect on growth of *C. trachomatis*, 156
- Co-trimoxazole and ampicillin compared in treatment of gonorrhoea, 236
- Co-trimoxazole and minocycline in treatment of prostatitis, 159*
- Co-trimoxazole in gonococcal urethritis, 236
- Counterimmunoelectrophoresis, detection of antibodies to *N. gonorrhoeae* by, 71*
- C-reactive protein in Jarisch-Herxheimer reaction in early syphilis, 154
- Crohn's disease, antibodies against LGV-type chlamydial in, 311
- Crohn's disease, chlamydial antibodies in, 312
- Cytomegalovirus, latency and activation of, 386*
- Diabetic patients, prevalence of yeasts in, 157*
- Disc agar diffusion susceptibility testing of yeasts, 157
- Drug resistance in *N. gonorrhoeae*, 71*
- Electron transport, effects of inhibitors in *N. gonorrhoeae*, 71*
- Erythromycin, in vitro activity against *N. gonorrhoeae*, 236
- Ether, topical, ineffectiveness in treatment of herpes genitalis, 73
- 5-fluorocytosine, effect on *C. albicans*, 385*
- 5-fluorocytosine, susceptibility of *C. albicans* to, correlation with serotype, 385*
- 5-fluorouracil on refractory genital warts, 311
- 5-fluorouracil urethral suppositories in genital warts, 238*
- Gonococcal antibodies and antigens, studies on circulating, 70
- Gonococcal arthritis, ESR in, 234
- Gonococcal conjunctivitis, laboratory acquired, 383*
- Gonococcal infections, disseminated, role of iron in, 70*
- Gonococcal membrane fractions, penicillin-binding in, 71*
- Gonococcal ophthalmia neonatorum, β -lactamase-producing *N. gonorrhoeae* causing, 234
- Gonococcal orogastric contamination, 155*
- Gonococcal pharyngitis and rheumatic fever in an adult, 383
- Gonococcal urethritis, asymptomatic, 155*
- Gonococcal urethritis, co-trimoxazole in treatment of, 236
- Gonococcal urethritis, pharmacokinetic determinants of penicillin cure of, 384
- Gonorrhoea, acridine orange-staining of smears for diagnosis of, 384*
- Gonorrhoea, bacampicillin in, 384
- Gonorrhoea, classes of antibodies in acute, 383
- Gonorrhoea, comparison of treatment with co-trimoxazole and ampicillin, 236
- Gonorrhoea, epidemiological treatment of, basis for, 236
- Gonorrhoea, IgA-cleaving protease in vaginal washings, 308
- Gonorrhoea in homosexual men, 155*
- Gonorrhoea in posthysterectomy patients, 154
- Gonorrhoea in pregnancy, 234
- Gonorrhoea patients, tracing and treating contacts of, 236*
- Gonorrhoea, pharyngeal, ampicillin in, 72
- Gonorrhoea, prevention with minocycline, 384
- Gonorrhoea, recent advances, 155*
- Gonorrhoea, screening for, 70*
- Gonorrhoea, treatment of, 385*
- Gonorrhoea, type-14 pneumococcal vaccine for prevention of, 384*
- Greenland, chancroid in, 1977-78, 311
- Haemophilus ducreyi*, antimicrobial sensitivity of, 73
- H. ducreyi*, hemin requirement, 73*
- H. influenzae*, in vitro activity of piperacillin and other β -lactamase antibiotics against, 384*
- H. vaginalis*, factors affecting isolation and identification, 312
- H. vaginalis* in non-specific vaginitis, 74*
- H. vaginalis*, role in non-specific vaginitis and treatment with metronidazole, 73
- H. vaginalis*, salient features of, 312
- Hawaii, non-gonococcal urethritis in, 72
- Hemagglutination tests for syphilis antibody, 70
- Hepatitis B, natural history and prevention re-examined, 312*
- Hepatitis B, prevention of, 386*
- Herpes, genital, and caesarian section, 385*
- Herpes, genital, ascending infection, 385*
- Herpes, genital, cellular immune response in, 157
- Herpes, genital, clinical course, diagnosis and evaluation of surfactant therapy, 310
- Herpes, genital, during pregnancy, 385
- Herpes, genital, ineffectiveness of topical ether in treatment of, 73
- Herpes, genital, levamisole treatment of, 72
- Herpes, genital, proctitis associated with, 158*
- Herpes, genital, semen in men with recurrent infections, 158
- Herpes, genital, treatment with photodynamic inactivation, 311*
- Herpes, genital, virus isolation versus immunofluorescent staining in diagnosis of, 158
- Herpes simplex, surgery for recurrence, 158*
- Herpes simplex virus, comparison of tests for antibody to, 385*
- Herpes simplex virus, differentiation between types 1 and 2 by antibodies, 385*
- Herpes simplex virus, immunoassay for antibodies to, 385*
- Herpes simplex virus, latency effect of pyran on, 385*
- Herpes simplex virus, restriction-enzyme technology to distinguish types 1 and 2, 385*
- Herpes simplex viruses, suppression of growth by cell-mediated, antibody and interferon mechanisms, 238*
- Herpes simplex virus type 2 antibodies in husbands of patients with cervical carcinoma, 73*
- Herpes simplex virus type 2 infection, antibody-mediated recovery from, 158*
- Herpes simplex virus type 2, organ culture model for cervical carcinoma, 385

- Herpes simplex virus type 2, relation to carcinoma of cervix, 385
- Herpes simplex virus type 2, relation to carcinoma of cervix: animal model for induction of latency, 73*
- Herpes simplex virus type 2 vaginal and systemic infections, comparison of macrophage inhibitory agents on, 238*
- Herpes virus fingerprinting, 311
- Herpes virus, immune control of latency, 385*
- Herpes virus vaccine, protection of guinea pigs, 238*
- Herpes viruses, persistence, reactivation and cell transformation, 385*
- Herpes viruses, replication and latency, 385*
- Homosexuals, ano-genital infection with *N. meningitidis*, 74
- Homosexuals, gonorrhoea in, 155*
- Homosexuals, liver disease in, 311
- Homosexuals, STD in, 312*
- Iron, role of, in disseminated gonococcal infections, 70*
- Jarisch-Herxheimer reaction, C-reactive protein in, 154
- Johannesburg, antibiotic sensitivity of *N. gonorrhoeae* in, 156*
- Levamisole treatment in recurrent herpes genitalis, 73
- Lipopolysaccharide (R-type) as a common antigen of *N. gonorrhoeae* (1) Immunizing properties, 71*
- (2) Hen antiserum in rapid slide test, 71*
- Liver disease in homosexuals, 311
- Metronidazole, single-dose treatment for *T. vaginalis*, 238*
- Metronidazole, *T. vaginalis* resistant to, 73*
- Metronidazole, treatment of *Haemophilus vaginalis* and role in non-specific vaginitis, 73
- Miconazole, clotrimazole and amphotericin B, in-vitro studies, 238
- Miconazole nitrate in candidal vaginitis, 385*
- Microbiota, oral, genital occurrence of, 158
- Microhemagglutination assay for antibodies to *T. pallidum*, 154*
- Minocycline and amphotericin B, combined activity against yeasts, 157
- Minocycline and co-trimoxazole in treatment of prostatitis, 159*
- Minocycline to prevent gonorrhoea, 384
- Mycoplasma, genital infections with, 237*
- Mycoplasma infection in pregnant rats, 157*
- Mycoplasmas, genital, relation to birthweight in primigravid women, 312
- Neisseria* confirmation using bicarbonate-containing carbohydrate medium, 235
- Neisseria gonorrhoeae* and *C. trachomatis* comparison of genital infections caused by, 237*
- N. gonorrhoeae* and *N. meningitidis*, differentiation with Congo-red disc and manganous chloride method, 235
- N. gonorrhoeae*, antibiotic sensitivity of, in Johannesburg, 156*
- N. gonorrhoeae*, antibiotic susceptibility of, 384*
- N. gonorrhoeae* antibodies, enzyme-linked assays for detection of, 234
- N. gonorrhoeae*, antigen-specific serotyping of, 235
- N. gonorrhoeae*, a restriction endonuclease from, 384*
- N. gonorrhoeae*, auxotypes and antibiotic susceptibility patterns from disseminated and local infections, 308
- N. gonorrhoeae*, auxotypes, phenotyping and epidemiology, 155
- N. gonorrhoeae*, binding of cholesterol by, 235*
- N. gonorrhoeae*, β -lactamase from, purification and properties of, 384*
- N. gonorrhoeae*, β -lactamase inhibition in, by sodium clavulanate, 235*
- N. gonorrhoeae*, β -lactamase-producing, in gonococcal ophthalmia neonatorum, 234*
- N. gonorrhoeae*, cell envelope phospholipase activity, 71*
- N. gonorrhoeae*, comparative susceptibility of penicillinase-positive and negative, to 30 antibiotics, 383
- N. gonorrhoeae*, comparison of antigenic heterogeneity by micro-immunofluorescence and serum bactericidal tests, 235
- N. gonorrhoeae*, comparison of rosamycin, erythromycin, penicillin, and tetracycline against, in vitro, 236
- N. gonorrhoeae*, complement-enhanced immunity to infection with, 384*
- N. gonorrhoeae*, conjugative plasmids in, 71*
- N. gonorrhoeae*, contribution of a TEM-1-like β -lactamase to penicillin resistance in, 71
- N. gonorrhoeae* CS7, cell envelope of, 308
- N. gonorrhoeae*, detection of antibodies by counter-immunoelectrophoresis, 71*
- N. gonorrhoeae*, differences in susceptibilities of virulent and avirulent strains to antimicrobial agents, 71
- N. gonorrhoeae*, effect of staphylococci on, 155
- N. gonorrhoeae*, effects of inhibitors on electron transport in, 71*
- N. gonorrhoeae*, effects of iron and culture filtrates on, 155
- N. gonorrhoeae*, fluorescent antibody in identification of, 155*
- N. gonorrhoeae*, hen fluorescein-labelled gonococcal antibody for confirmation of, 383
- N. gonorrhoeae*, identification and penicillinase testing, on cultures on New York City Medium, 71*
- N. gonorrhoeae*, identification by slide agglutination, 155*
- N. gonorrhoeae*, identification in liquid fermentation medium, 383
- N. gonorrhoeae*, identification of basic extractable compounds produced by, 71*
- N. gonorrhoeae*, inhibition of thiamphenicol and chloramphenicol, 235*
- N. gonorrhoeae*, in-vitro activity of a new cephalosporin against, 384*
- N. gonorrhoeae*, in vitro activity of piperacillin and other β -lactamase antibiotics against, 384*
- N. gonorrhoeae*, lipopolysaccharide (R-type) as a common antigen of, (1) Immunizing properties, 71*
- (2) Hen antiserum in rapid slide test, 71*
- N. gonorrhoeae*, multiple drug resistance in, 71*
- N. gonorrhoeae*, pathogenicity of colonial type 5, 71*
- N. gonorrhoeae*, penicillinase-producing in New York, 384*
- N. gonorrhoeae*, penicillin-resistance associated with patient variables, 236*
- N. gonorrhoeae*, pharyngeal coloniser or pathogen?, 384*

- N. gonorrhoeae*, plasmid-mediated chromosomal gene transfer in, 71*
- N. gonorrhoeae*, purification of immunoglobulin A1 protease, 235
- N. gonorrhoeae*, rapid microcarbohydrate test for confirmation of, 234
- N. gonorrhoeae*, relationship of *H. influenzae* plasmid to R factors from, 384*
- N. gonorrhoeae*, ribosomal fractions, mitogenic activity elicited by, 71*
- N. gonorrhoeae*, serodiagnosis, of nuclease enhancement of specific cell agglutination, 384*
- N. gonorrhoeae*, serology of demonstration of strain-specific antigens, 308
- N. gonorrhoeae*, susceptibility to cefoxitin sodium, 156
- Neisseria*, identification of, comparison of radiometric and conventional methods, 71*
- N. meningitidis*, ano-genital infection in homosexuals, 74
- N. meningitidis*, identification of basic extractable compounds produced by, 71*
- Neisseria* species, three methods compared for identification of, 384*
- Neisseriae*, antibody-cleaving, 155*
- Nephrotic syndrome, associated with endemic syphilis, 70
- Neurosyphilis today, 307
- New York, penicillinase-producing *N. gonorrhoeae* in, 384*
- Non-gonococcal urethritis, diagnostic aetiology, 308
- Non-gonococcal urethritis, examination of patients and contacts for *C. trachomatis* and *U. urealyticum*, 236
- Non-gonococcal urethritis in Hawaii, 72
- Non-gonococcal urethritis, recent advances, 155*
- Non-gonococcal urethritis, serological response of patients with, to organism of contagious equine metritis, 310
- Non-specific vaginitis: role of *Haemophilus vaginalis* and treatment with metronidazole, 73
- Non-specific vaginitis, *Haemophilus vaginalis* in, 74*
- Oedema, penile, venereal, 312*
- Papanicolaou smears, abnormal, cervicitis and chlamydiae, 310*
- Papules, pigmented penile, with carcinoma *in-situ* changes, 238*
- Penicillin binding by gonococcal membrane fractions, 71*
- Penicillin cure of gonococcal urethritis, pharmacokinetic determinants of, 384
- Penicillin, in-vitro activity against *N. gonorrhoeae*, 236
- Penicillin resistance in *N. gonorrhoeae*, contribution of a TEM-1-like beta-lactamase to, 71
- Perihepatitis associated with salpingitis in adolescents, 154
- Piperacillin, in-vitro activity against *N. gonorrhoeae*, and *H. influenzae*, 384*
- Plasmid-mediated chromosomal gene transfer in *N. gonorrhoeae*, 71*
- Plasmids, conjugative, in *N. gonorrhoeae*, 71*
- Pneumonia and *C. trachomatis* in infants, two cases, 309
- Pneumonia due to *C. trachomatis* in baboons, histopathology, 309
- Pneumonitis, chlamydial, 73*, 309
- Podophyllin poisoning, 158
- Post-gonococcal urethritis in homosexual and heterosexual men, roles of *C. trachomatis* and *U. urealyticum*, 308
- Posthysterectomy patients, gonorrhoea in, 154
- Proctitis associated with genital herpes, 158*
- Prostatitis, co-trimoxazole, and minocycline in treatment of, 159*
- Pyran, effect of, on latency of herpes simplex virus, 385*
- Reiter's syndrome, association with *Chlamydia* infection, 237
- Rheumatic fever and gonococcal pharyngitis in an adult, 383
- Rosamicin, in vitro activity against *N. gonorrhoeae*, 236
- Rosamicin, sensitivity of *C. trachomatis* to, 156
- Salpingitis, acute, bacteriology of, 312
- Salpingitis, experimental, in monkeys, from *C. trachomatis*, 310*
- Semen in men with recurrent genital herpes, 158
- Sexual assault; victim's psychology and related problems, 74*
- Skin, late benign syphilis of, 70*
- Sodium clavulanate, inhibition of β -lactamase in *N. gonorrhoeae*, 235*
- Staphylococci, effect on *N. gonorrhoeae*, 155
- STD, epidemiological approach to control of, 386*
- STD in children, 386*
- STD in homosexuals, 312*
- Syphilis and yaws, 382 bis*
- Syphilis antibody, hemagglutination tests for, 70
- Syphilis, comparison of haemagglutination treponemal test with other serological tests, 233
- Syphilis, congenital, transplacental transmission of spirochaetes, 234*
- Syphilis, early, C-reactive protein in Jarisch-Herxheimer reaction, 154
- Syphilis, early latent, evaluation of treatment, 233*
- Syphilis, endemic, associated with nephrotic syndrome, 70
- Syphilis, hearing loss in, 382*
- Syphilis, late benign of skin, 70*
- Syphilis secondary, and Bell's palsy, 307
- Syphilis, serology, ELISA test, 233
- Syphilis, serology, false-positive reactions in, 382*
- Syphilis, serology, fluorescent treponemal antibody absorption test, 382
- Syphilis, serology, Rappaport rapid plate test, 234*
- Syphilis, serology, Syphla-Chek test, 382
- Syphilis, serology, TPI test, 233*
- Tetracycline, in-vitro activity against *N. gonorrhoeae*, 236
- Tetracycline-resistant strains of *Ureaplasma urealyticum*, incidence of, 72
- Treponema*, genetics of, relationship of *T. pallidum* and *T. hyodysenteriae*, 234*
- Treponema pallidum* and *T. pertenue*, comparative behaviour of virulent strains, 382
- T. pallidum*, capacity for DNA synthesis, 308
- T. pallidum*, direct immunofluorescence of antibodies to, in rabbit syphilomas, 154
- T. pallidum*, glucose incorporation by, 382*

- T. pallidum*, influence of oxygen tension, sulphhydryl compounds and serum on motility and virulence, 307
- T. pallidum*, in-vitro activity of cefuroxime against, 70*
- T. pallidum*, microhemagglutination assay for antibodies to, 154*
- T. pallidum*, mucopolysaccharides of, 383*
- T. pallidum*, radiolabelling with precursors for protein and RNA biosynthesis, 154*
- T. pallidum*, relationship to acidic mucopolysaccharides, 383*
- T. pallidum*, respiration and oxidative phosphorylation in, 154*
- T. pallidum*, surface mucopolysaccharides of, 382*
- Trichomonas vaginalis* infections, evaluation of indirect hemagglutination techniques in study of, 237
- T. vaginalis* resistant to metronidazole, 73*
- T. vaginalis*, single-dose metronidazole in treatment of, 238*
- T. vaginalis*, single-dose treatment with benzylmetronidazole, 238*
- T. vaginalis*, strain resistant to metronidazole and other 5-nitro-imidazoles, 310*
- Tunisia, antichlamydial antibody in tears and sera in, 157*
- Ulcerative colitis, chlamydial antibodies in, 312
- Ureaplasma urealyticum*, cell fractions and enzymatic activities of, 237*
- U. urealyticum*, cultivation in continuous culture, 310*
- U. urealyticum*, difficulties with isolation and identification of, 157
- U. urealyticum*, effect of ammonium ion and osmotic pressure on growth of, 73*
- U. urealyticum*, examination for, in patients with non-gonococcal urethritis, 236
- U. urealyticum*, incidence of tetracycline-resistant strains, 72
- U. urealyticum*, role in post-gonococcal urethritis in homosexual and heterosexual men, 308
- U. urealyticum*, serological typing in urethritis patients, 237
- U. urealyticum*, urethral infection of chimpanzees by, 72
- Urethritis, clinical and microbiological investigation of men with, 310
- Vaginitis, 386*
- Vaginitis, non-specific, amine content of vaginal fluid in, 386*
- Vaginitis, quantitative bacteriology of vaginal flora, 386
- Warts, genital and skin, wart virus antibodies in patients with, 311
- Warts, genital, 5-fluorouracil on refractory, 311
- Warts, genital, 5-fluorouracil urethral suppositories in genital warts, 238*
- Warts, genital, in a mother and infant, 158*
- Warts, genital, incidence of associated infections, 158
- Yaws and syphilis, 382 bis*
- Yeasts, combined activity of minocycline and amphotericin B against, 157
- Yeasts, disc agar diffusion susceptibility testing of, 157
- Yeasts, prevalence in diabetic patients, 157*

Index to authors of articles abstracted, 1979

*Indicates that only the title is given.

Adam, E., 311*
 Adams, M. R., 382
 Albritton, W. L., 73*
 Alessi, E., 233
 Alexander, E. R., 156, 308, 309
 Allen, S. D., 308
 Alley, C. C., 71*
 Alpert, S., 237, 310*
 Amshel, A. L., 158*
 Amstey, M. S., 385*
 Appelbaum, P. C., 384*
 Arko, J. R., 384*
 Aronson, I. K., 154
 Arth, C., 309*
 Arum, E. S., 310
 Ashton, F. E., 71*, 155*, 234, 383, 384*
 Auger, P., 385*
 Austin, T. W., 155*

Babb, R. R., 312*
 Babiak, L. A., 385*
 Bailey, R. K., 312
 Baker, C. N., 235*, 384*
 Baker, D. A., 385
 Banatvala, J. E., 311
 Barbieri, J. T., 382*
 Barling, R. W. A., 74*
 Barlow, D., 72
 Barrada, M. I., 234
 Baseman, J. B., 308
 Bawdon, R. E., 233
 Becker, F. A., 382*
 Beckett, K. M., 157
 Beezer, A. E., 238
 Bengtsson, S., 384
 Benjamin, D., 309
 Bergström, S., 71
 Berman, L., 386*
 Biddle, J. W., 308
 Biswas, G., 71*
 Blackman, E., 71*
 Blake, H. M., 308*
 Blake, M. S., 235
 Blenk, B., 155*
 Blenk, H., 155*
 Blumstein, G., 234
 Bosmans, E., 384*
 Bowie, W. R., 156, 308
 Boyce, J. T., 309
 Braff, E. H., 155*
 Breinig, M. C., 385*
 Brickman, F., 154

Brodeur, B. R., 71*, 234
 Brooks, J. B., 71*
 Brown, W. J., 308
 Bruins, S. C., 383*
 Buchanan, T. M., 235, 386*
 Bull, J., 311
 Bullock, J. P. jnr., 312
 Burtchell, S., 73*
 Bushell, A. C., 156
 Buzzetti, C., 233

Cacciapuoti, A. F., 71*
 Carbone, J., 72
 Carbonetto, C., 155*
 Carr, M. C., 310*
 Catalan, F., 73*
 Centifanto, Y. M., 158
 Chamberlain, M. A., 238*
 Chan, M. A., 71*
 Chandler, J. E., 154
 Chang, T.-W., 73*
 Cherian, S., 383
 Chiang, W. T., 73, 309
 Cho, C. T., 158
 Choudhary, G., 71*
 Chow, A. W., 312
 Christiansen, S., 233*
 Christman, J. L., 384*
 Clanton, D. J., 384*
 Clune, P. F., 385*
 Cohen, M. I., 154
 Colaert, J., 384*
 Coleman, J. C., 311
 Cooke, C. L., 312*
 Cooter, N. B. E., 385
 Corey, L., 73, 157, 310
 Coserne, R. F., 238
 Cox, C. D., 154*, 382*
 Crowley, M., 386*
 Cruce, D. C., 154*
 Culp, D. A., 238*
 Cummings, W. A., 383
 Curry, J. P., 158*

D'Amato, R. F., 384*
 Daghfous, T., 157*
 Danielsson, D., 308
 Dans, P. E., 70, 154
 Darougar, S., 156, 157* (bis)
 Davis, L. E., 307
 Deinhardt, F., 312*
 Delouvois, J., 312

Deture, F. A., 158
 Dicaprio, J. M., 72
 Diena, B. B., 71* (bis), 155*, 234, 383, 384
 Digranes, A., 310*
 Dillon, J. R., 235*, 236
 Dines, R. J., 156
 Drylie, D. M., 158
 Duck, P. D., 235*, 236
 Dumas, C., 385*
 Durfee, M. A., 73
 Dwyer, R. St. C., 157*
 Dychman, J. D., 382
 Dykers, J. R., 238*

Edlingber, E., 73*
 Edwards, L. E., 234
 Eftaiha, M. S., 158
 Ehret, J. M., 74
 Eickhoff, T. C., 74
 Eidus, L., 235*, 236
 Ellis, W. R., 311
 El-Sheikh, H., 156, 157*
 Elwing, H., 385*
 Embil, J. A., 158*, 309*
 Erikson, G., 384
 Eriquez, L. A., 384*
 Esposito, A., 386*
 Evans, B. A., 311
 Evans, E. G. V., 157*
 Evans, R. T., 72

Falkow, S., 71*
 Farshy, C. E., 154*
 Fasse, R. J., 236
 Feeley, J. C., 154*
 Felman, Y. M., 384*, 386*
 Feng, K. K., 158
 Fieldsteel, A. H., 234*, 382*
 Finkelstein, R. A., 70*
 Fish, E. N., 73*, 385 (bis), 385*
 Fitzgerald, T. J., 307, 382* (bis)
 Fitzl, D. M., 383*
 Fiumara, N. J., 73, 233*, 310
 Fluker, J. L., 311
 Floro, C. N., 386
 Forsum, U., 158, 384*
 Forsyth, P. S., 73, 386*
 Frank, G., 72
 Fraser, C. E. O., 385*
 Freundt, E. A., 310*
 Friberg, J., 237*

- Friedman, H., 71*
 Frosner, G. G., 312*
 Fuccillo, D. A., 385*
 Fudenberg, H. H., 238*

 Gaafar, H. A., 71*
 Gaarslev, K., 311
 Gaillard, M. C., 156*
 Gale, J. L., 309
 Gartner, L., 73*
 Gatenbein, B., 382
 Geary, I., 383
 Ghinsberg, R., 234
 Giddens, W. E. jnr., 309
 Glauser, M. P., 159*
 Gleckman, R., 386*
 Goette, D. K., 70*
 Glynn, A. A., 383
 Goldbloom, R. B., 310*
 Goldner, M., 71*
 Goust, J. M., 238*
 Graves, J. O., 235
 Grayston, J. T., 237*
 Greenwood, J. R., 312*
 Greenwood, R. J., 307
 Grossman, M., 309* (bis)

 Hafiz, S., 71*, 235, 383
 Hager, W. D., 234
 Hakanson, E. Y., 234
 Hall, W. H., 383
 Hallen, A., 384*
 Hamann, A. A., 234
 Hammond, G. W., 73 (bis)
 Handsfield, H. H., 155*
 Hanna, L., 310*
 Haram, K., 310*
 Harris, D. L., 234*
 Harrison, H. R., 309
 Harrison, R. F., 312
 Harrison, W. O., 384
 Hebel, B. H., 308*
 Heise, H., 73*
 Herrmann, K. L., 308
 Hjelm, E., 158*
 Hobson, D., 156
 Hoeprich, P. D., 157
 Hofmann, R., 73*
 Holderbach, J., 71*
 Holmberg, K., 158
 Holmes, K. K., 70*, 73 (bis), 155, 157, 308, 308*, 310, 386*
 Holt, J., 309*
 Hoofugle, J. H., 386*
 Hooper, R., 384
 Horger III, E. O., 238*
 Hunter, E. F., 382
 Hurd, J. K., 386*
 Hurley R., 312
 Hurt, W. G., 312*
 Ison, C. A., 383

 Jacobs, N. F., 308, 310
 Jaffe, H. W., 70, 236*, 384
 Jahn, G., 384*
 Jansson, E., 236
 Jawetz, E., 310*
 Jeansson, S., 385*
 Jefferiss, J., 382*
 Jenkin, H. M., 154*
 Jenkins, T., 386*
 Jennings, R., 71*
 Jephcott, A. E., 71*
 Johnson, A. L., 72
 Johnson, L. D., 385*
 Johnson, R. C., 382* (bis)
 Joly, J., 385*
 Joncas, J. H., 385*
 Jones, B. R., 156, 157*
 Jones, O. G., 70
 Jordan, W. P., 312*
 Josey, W. E., 385*
 Judson, F. N., 74, 236, 236*, 312*
 Junge, W., 155*

 Kafman, R., 234*
 Kallings, L.-O., 384
 Katz, H. I., 238*
 Kaufman, H. E., 158
 Kaufman, R. H., 311*
 Kaulfers, P. M., 384*
 Keeling, P. W. N., 311
 Kellogg, D. S., jnr, 71*
 Kenimer, E. A., 71*
 Kenny, G. E., 73*
 Kindmark, C.-O., 154
 Kinghorn, G. R., 158
 Kirk, C. S. G., 386*
 Klaus, B. D., 154
 Klein, A., 157*
 Knapp, J. S., 155
 Knopf, O., 74*
 Koornhof, H. J., 156*
 Kousa, M., 236, 237
 Kraus, S. J., 308, 310
 Krugman, J., 312*
 Kuberski, T., 72, 237
 Kumagai, K., 238*
 Kundsinn, R. B., 157
 Kunkel, M., 73*
 Kuo, C. C., 73*
 Kwapinski, E., 70
 Kwapinski, G., 70

 Lapp, D. F., 71*
 Larsen, L., 311
 Larsen, S. A., 70, 154*, 382
 Lassus, A., 236, 237
 Laufs, R., 384*
 Lawrence, R. B., 384*
 Lawson, J. W., 155
 Lee, C. K., 156
 Lee, Y.-H., 310
 Lees, A. J., 307

 Leitch, R. A., 383
 Lent, B., 155*
 Le Roux, F. B., 70
 Levi, A. J., 312
 Levin, M. J., 157
 Levison, M. E., 386
 Lew, M. A., 157
 Lian, C. J., 73 (bis)
 Licata, R. L., 237*
 Liddle, J. A., 71*
 Lim, D. V., 71
 Ling, C.-M., 312*
 Litt, I. F., 154
 London, W. T., 72
 Lonsdale, D. M., 385*
 Lorincz, A. L., 154
 Lunceford, C. D., 237
 Luxon, L., 307
 Lykke-Olesen, T., 311
 Lysko, P. G., 154*

 Maccani, J. E., 383
 McClelland, C. Q., 386*
 McComb, D. E., 237
 McCormack, W. M., 237, 310*, 385*
 MacDonald, S. W., 309*
 McEntegart, M. G., 71*, 235
 McGeorge, N. B., 238*
 McGinley, D., 238*
 Madden, D. L., 385*
 Magee, L. A., 235
 Maltz, A. B., 236
 Malysheff, C., 71*, 155*
 Manuel, F. R., 158*
 Mårdh, P.-A., 309, 310* (bis)
 Mark, J. A., 235
 Marshall, J. R., 312
 Martin, J. E., 384
 Masover, G., 310*
 Mathur, S., 238*
 Matin, A., 310*
 Mayheur, S. R., 385*
 Medoff, G., 384*
 Meingassner, J. G., 73*, 310*
 Meir, E., 234*
 Meller, B. R., 310*
 Melnick, J., 311*
 Melsen, F., 310*
 Miao, R. M., 234*
 Michel, M. F., 311
 Miles, R. J., 238
 Milleman, L. A., 238*
 Miller, J. M., 235*
 Miller, J. N., 307
 Miller, R. D., 235*
 Miller, R. V., 384*
 Minassian, D., 156
 Mirkovic, R. R., 311*
 Moberg, I., 384
 Mogerley, S., 308*
 Mohammed, W., 71*

- Moller, H., 154
 Monif, G. R. G., 385*
 Montplaisir, S., 386*
 Morahan, P. S., 238*, 385*
 Morriss, D. M., 155
 Morse, S. A., 71*, 235*, 308*
 Murray, B. K., 385*
 Murray, D., 234*
 Murray, E. S., 238
 Murray, P. R., 384*
 Murray-Lyon, I. M., 311
 Mushahwar, I. K., 312*

 Nahmias, A. J., 385*
 Naot, Y., 157*
 Natsios, G. A., 386*
 Nayyar, K., 72
 Nichols, J. C., 308*
 Nichols, R. L., 237, 238
 Nikitas, J. A., 386*
 Nilsson, L. A., 385*
 Nishimura, R., 72
 Nisson, P. E., 238
 Nord, C.-E., 158
 Norlander, L., 71
 Normark, S., 71
 Norqvist, A., 71
 Norris, S. J., 307
 Norrod, P., 155

 Oates, J. K., 158*
 Oberender, H., 73*
 O'Brien, P. C., 237*
 Odds, F. C., 157*
 Odugbemi, T. O., 235, 383
 Oetgen, W. J., 73*
 Ohye, R., 72
 O'Morain, C. S., 312
 Oriel, J. D., 72
 Orrison, L. H., 382
 Orsolini, P., 70*
 Ouizman, E., 73*
 Overby, L. R., 312*
 Oxman, M. A., 385*
 Ozere, R. L., 309*

 Paavonen, J., 72, 236
 Pahor, A. L., 382*
 Pang, R., 234*
 Papsin, F. R., 73*, 385 (bis), 385*
 Parreno, A., 157
 Patten, V., 312
 Pattison, F. L. M., 155*
 Paulson, D. F., 159*
 Payne, S. M., 70*
 Peacock, W. L., 384*
 Pedersen, T. G., 311
 Pender, B. J., 382
 Perez, R., 310*
 Perkins, R. L., 236
 Perry, M. B., 71*, 72, 155*, 383, 384
 Persson, K., 154
 Peter, C. R., 233, 382*

 Peterson, R. G., 158
 Pettersen, G. R., 237*
 Pheifer, T. A., 73
 Phillips, I., 156
 Pickett, M. J., 312*
 Piket-van Ulsen, J., 311
 Piot, P., 384*
 Plotkin, S. A., 385
 Polak, A., 385
 Pollock, H. M., 73
 Posalaky, Z., 238*
 Poulin, S., 157
 Prakash, S. S., 385*
 Prescott, C. E., 70*
 Prior, R. B., 236
 Prytula, A., 234
 Purcell, R. H., 72
 Pyrhönen, S., 311

 Quach, R., 386

 Rajan, V. S., 234*
 Rattray, M., 310
 Rätz, K.-H., 73*
 Reeve, P., 72
 Reeves, W. C., 73, 157, 310
 Relland, T. L., 238*
 Rein, M. F., 308
 Remington, M., 73
 Remington, M. A., 310
 Reynolds, G., 308
 Reynolds, G. H., 236*, 384
 Reynolds, J., 72
 Richmond, S., 237
 Riggsby, W. S., 384*
 Ripa, K. T., 309, 310* (bis)
 Roberts, M., 71*
 Robinson-Browne, R. M., 156*
 Rodriguez, G. E., 312*
 Rodriguez, W. J., 71*
 Rogolsky, M., 155
 Romano, N., 237*
 Ronald, A. R., 73 (bis)
 Roos, R. F., 238*
 Rosemond-Hornbeak, H., 158*
 Rosenthal, L. J., 385*
 Rosenthal, R. O., 310
 Rosner, B., 310*
 Rothenberg, R., 382*
 Rouse, B. T., 385*
 Rumack, B. H., 158
 Ruskin, J., 236
 Ryan, A., 71* (bis)

 Sacchi, G., 233
 Saikku, P., 72, 236, 237
 Salkon, J. B., 74*
 Sandok, P. L., 154*
 Sandström, E., 308, 384
 Satoh, J., 238*
 Sattler, F. R., 236
 Saubolle, M. A., 157
 Sayed, I. A., 73*

 Saz, A. K., 71*
 Schachter, J., 309* (bis)
 Schalla, W. O., 384*
 Schapira, M., 159*
 Schieri, E. A., 383
 Schneider, J., 156*
 Schonberg, I. L., 158*
 Schoolnik, G. A., 155
 Schroder, J. A., 71*
 Schuller, J. L., 311
 Schweid, A., 310
 Scriba, M., 238*
 Semine, D. Z., 237
 Seth, P., 385*
 Sharf, M., 157*
 Shelley, W. B., 158*
 Shepard, M. C., 237
 Shmizu, F., 238*
 Short, H. B., 308
 Shulman, J. A., 308
 Siboulet, A., 73*
 Siegel, M. S., 384*
 Simmons, P. D., 311
 Sizemore, R. C., 235*
 Sladowski, C., 386
 Slater, G. E., 158
 Sly, D. L., 72
 Smith, R. F., 312
 Smith, T. F., 156, 237*
 Sng, E. H., 234*
 Snyder, R., 384*
 Soltani, K., 154
 Sox, T. E., 71*
 Sparling, P. F., 71*
 Spector, S., 309*
 Sperry, S., 307
 Squires, S., 157*
 Stalder, H., 385*
 Stargel, M. D., 308
 Steurer, F. J., 384*
 Stolz, E., 311
 Stout, J. G., 382*
 Strauss, R. R., 71*
 Studer, J.-P., 159*
 Suffield, W. E., 385*
 Svensson, L., 309
 Swanson, J., 235, 308*
 Swartz, S. L., 308
 Sweet, R., 309*
 Sykes, J. A., 307

 Tabatabai, M. P., 383
 Tada, M., 238*
 Tassi, G. C., 233
 Taylor, C. E. D., 310
 Taylor, M. A. R., 157*
 Taylor-Robinson, D., 72 (bis),
 310, 312
 Teh, L. B., 234*
 Teschner, V., 384*
 Thayer, J. D., 384
 Thelin, I., 310*

Thomas, B. J., 72, 312
Thompson, M. A., 382*
Thompson, M. S., 233
Thompson, R. P. H., 311
Thompson, S. E., 308
Thornsberry, C., 155, 235*, 236, 308, 384*
Turner, J., 73*, 310*
Tight, R. R., 383*
Tobin, S. M., 73, 385 (bis), 385*
Tolentino, E., 310
Trappier, B., 386*
Treharne, J. D., 156, 157*
Trestman, I., 386

Ursi, J.-P., 384*
Uyeki, M., 386*

Valcurone, G., 233
van Buuren, A. J., 70
van Dyck, E., 384*
Veeken, I. V. D., 311
Vesterinen, E., 236

von Krogh, G., 311
von Schmidt, B., 309
Vontver, A., 73, 310
Voss, J. L., 312

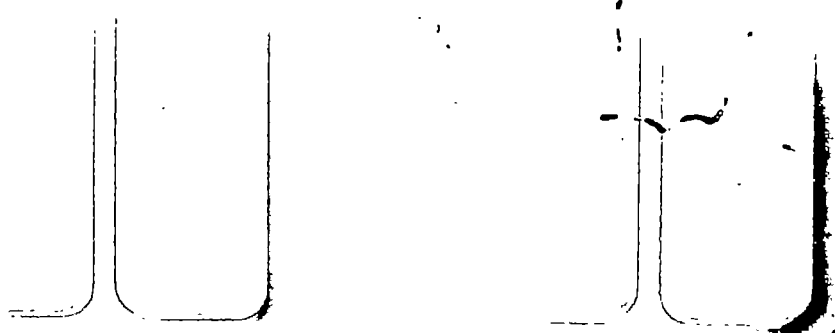
Wain, W. H., 385*
Wales, J. K., 157*
Wallace, R., 71* (bis), 155*, 383
Wallin, J., 158, 384, 384*
Wallmark, G., 384
Wang, S.-P., 235, 237*
Warren, W. J., 235*
Washton, H. E., 156
Webb, C. J., 70
Weed, L. A., 237*
Wegener, W. A., 71*
Weimar, G. W., 238*
Wende, R. D., 382
Wentworth, B. B., 233
Weström, L., 309*
White, R. D., 159*
Wibbelsman, C. J., 155*
Wiebenga, N., 72
Wiesner, P. J., 155, 236* (bis), 384

Willcox, J. R., 311
Williams, C. N., 158*
Williams, R. P., 155, 382
Wilson, D. C., 382*
Wilson, D. L., 233
Wilson, W. D., 73, 385, 385*
Wilt, J. C., 73 (bis)
Winter, C., 73
Wolf, F. C., 236*
Wong, K. H., 384* (bis)
Wong, W., 308*
Woodland, R. M., 157*
Wright, R. A., 312*

Xerri, L., 70*

Young, D. C. T., 234
Young, F. E., 308, 308*
Young, H., 71*
Young, R. L., 311*

Zeidi, A. A., 236*, 308, 384
Zemaityte, D. E., 386*
Zinner, S. H., 237



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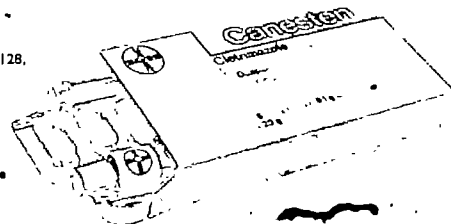
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Contents

Oxygen concentration of dissolved oxygen for the survival of virulent *Treponema pallidum* under conditions of low oxidation-reduction potential

S. GRAVES AND T. BILLINGTON (Australia) page 387

Susceptibility of rabbits to *Treponema pallidum* after infection with *Mycobacterium bovis*

S. GRAVES (Australia) page 394

Limited protection of rabbits against infection with *Treponema pallidum* by immune rabbit sera

S. GRAVES AND J. ALDEN (Australia) page 399

Reverse passive haemagglutination test for the rapid identification of *Neisseria gonorrhoeae* and detection of penicillinase production

R. MUNRO AND R. MALLON (Australia) page 404

Prevalence of rectal and pharyngeal infection in women with gonorrhoea in Sheffield

G. R. KINGHORN AND S. RASHID (Gt Britain) page 408

Comparison of minocycline and ampicillin in gonococcal urethritis

M. A. WAUGH, E. MARY COOKE, B. B. G. NEHAUL, AND JANET BRAYSON (Gt Britain) page 411

Chlamydia trachomatis genital infections

F. D. GHADIRIAN AND H. G. ROBSON (Canada) page 415

Antichlamydial antibodies in pelvic inflammatory disease

P. D. SIMMONS, T. FORSEY, R. N. THIN, J. D. TREHARNE, S. DAROUGAR, F. LANGLET, AND P. K. PANDHI (Gt Britain) page 419

Chlamydia trachomatis infection of the Fallopian tubes

B. R. MØLLER, L. WESTRÖM, S. AHRONS, K. T. RIPA, L. SVENSSON, C. von MECKLENBURG, H. HENRIKSON AND P.-A. MÅRDH (Denmark and Sweden) page 422

Prevalence of nine different micro-organisms in the female genital tract

K. PERSSON, KRISTINA PERSSON, H. HANSSON, B. BJERRE, L. SVANBERG, T. JOHNNSSON, AND A. FORSÖREN (Sweden) page 429

Effect of hysterectomy on genital infections

F. N. JUDSON AND M. A. RUDER (USA) page 434

Rising incidence of chancroid in Rotterdam

K. C. NAYYAR, E. STOLZ, AND M. F. MICHEL (Holland) page 439

Effect of injections of small doses of human fibroblast interferon into genital warts

G. M. SCOTT AND G. W. CSONKA (Gt Britain) page 442

Perigenital cutaneous schistosomiasis

F. A. B. ADEYEMI-DORO, A. O. OSOBA, AND T. A. JUNAID (Nigeria) page 446

Correspondence page 450

Correction page 450

Book review page 451

Index page 451